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(71) Applicant: CHEMEX PHARMACEUTICALS, INC. [US/US]; 1401 17th Street, Suite 850, Denver, CO 80202 (US).

(72) Inventors: JORDAN, Russell, T. ; 1809 Indian Meadows Lane, Fort Collins, CO 80525 (US). ALLEN, Larry, M. ; 450 A Josephine Street, Denver, CO 80206 (US).

(74) Agents: LEMPEL, Paul et al.; Kenyon & Kenyon, One Broadway, New York, NY 10004 (US).

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(54) Title: PHARMACOLOGICALLY ACTIVE COMPOSITIONS OF CATECHOLIC BUTANES WITH ZINC

(57) Abstract

Pharmacologically active compositions of catecholic butanes and the use thereof in the treatment of diseases and disorders of the skin, and their use as antibacterial and antifungal agents. The compositions are also useful in the treatment of benign, premalignant and malignant solid tumors, especially those of the skin. The compositions according to the invention may also include ionic zinc and other metal ions which enhance the effect of the catecholic butane compounds.

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PHARMACOLOGICALLY ACTIVE COMPOSITIONS OF CATECHOLIC BUTANES WITH ZINC

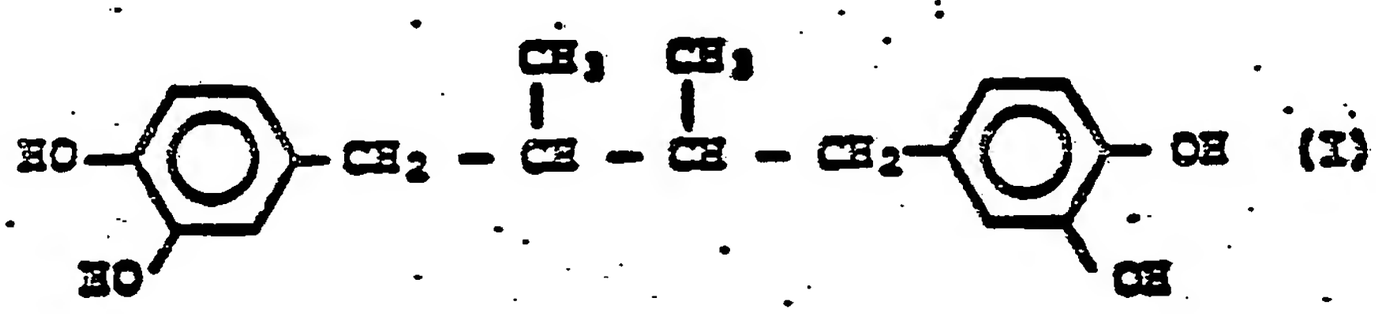
Field of the Invention

This invention relates to pharmacologically active compositions of catecholic butanes and ionic zinc and to the use thereof in the treatment of diseases and disorders of the skin, and to their use as antibacterial and antifungal agents. They are also useful in the treatment of benign, premalignant and malignant solid tumors, especially those of the skin.

Background

Mammals can be affected with a wide variety of skin disorders including bacterial and fungal infections and benign, premalignant and malignant growths. The systemic application of antibiotics has been commonly used as a treatment for bacterial infections in conditions such as acne and osteomyelitis. More recently, the topical use of certain antibiotics has been reported. For example, erythromycin in combination with zinc acetate has been reported as being useful in the topical treatment of

acne. Many other chemicals have been reported as having antimicrobial activity. Some of these, such as the meso form of nordihydroguaiaretic acid [meso-1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane] which occurs naturally, have been used as folk remedies. Unless otherwise indicated, NDGA is used herein to refer to the meso form of nordihydroguaiaretic acid. NDGA is a principal component in the creosote bush which has been used to make a tea used as a folk remedy for colds, rheumatism and other ailments for centuries. However, most of these chemicals including NDGA have not proven to be successful in the treatment of conditions such as acne and osteomyelitis. The general structure for nordihydroguaiaretic acid is given in Formula (I).



(I)

Methods of treating premalignant and malignant growths of the skin have often been traumatic. A common method of treating disorders such as actinic keratosis has been the application of liquid nitrogen to destroy the affected tissue. Epidermal tumors are commonly treated by physical removal through surgery. A method which has been used in the past is chemosurgery through the application of escharotic or fixative chemicals such as zinc chloride. This has not been found to be particularly effective because of the physical discomfort associated with the use of such materials. It also has the disadvantage of destroying both healthy tissue and the diseased tissue.

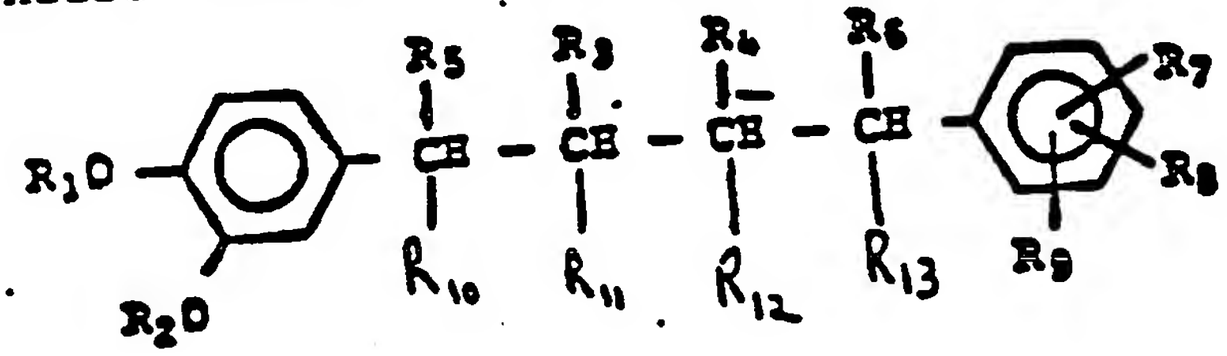
The use of known antitumor drugs has not been found to be particularly effective in the treatment of skin tumors since these drugs are commonly applied systemic-

ally resulting in substantial side effects due to their toxicity. NDGA was reported as providing a positive result against malignant melanoma. However, a clinical study was conducted by Smart, et al. in which human cancer patients ingested either a tea made from the creosote bush or doses of pure NDGA. This study indicated that neither NDGA nor the tea were effective anticancer agents and in some cases caused a stimulation of tumor cell growth. C. R. Smart, et al. Rocky Mountain Medical Journal, Nov. 1970, pp. 39-43. This confirmed earlier screening studies of NDGA conducted by the Cancer Chemotherapy National Service Center which obtained negative results when NDGA was tested against several types of cancer cells.

Surprisingly, we have found that nordihydroguaiaretic acid in a pharmaceutical composition containing ionic zinc is effective in treating disorders of the skin including bacterial infections which occur in acne and in osteomyelitis when applied to the situs of the disorder. Such compositions are also effective in treating benign, premalignant and malignant growths of the skin without the detrimental side effects associated with chemosurgical techniques, when applied topically to or injected into the growth.

Summary of the Invention

In a compositional aspect, this invention relates to pharmaceutical compositions comprising a preferred catecholic butane of formula



(II)-

wherein R_1 and R_2 are independently H, lower alkyl or lower acyl;

R_3, R_4, R_5 and R_6 are independently H or lower alkyl;

R_7, R_8 and R_9 are independently H, hydroxy, lower alkoxy or lower acyloxy;

R_{10}, R_{11}, R_{12} and R_{13} are independently H or lower alkyl.

Lower alkyl is intended to generally mean C_1-C_6 alkyl, and preferably R_3 and R_4 are C_1-C_3 alkyl. Lower acyl is intended to generally mean [C_1-C_5] acyl, with [C_2-C_6] acyl being preferred. It will be appreciated by those skilled in this art that Formula II is directed to both the phenolic compounds and the conventional esters and ethers thereof.

In another composition aspect, this invention relates to pharmaceutical compositions adapted for topical administration comprising, in admixture with a pharmaceutically acceptable carrier, a mixture of (i) a catecholic butane of Formula (II) and (ii) a pharmaceutically acceptable source of ionic zinc.

In preferred composition aspect, this invention relates to such compositions, adapted for topical application to a situs or injection into the interior of the situs, comprising nordihydroguaiaretic acid and a source of ionic zinc, and to such compositions in combination with a pharmaceutically acceptable carrier.

In a method of use aspect, this invention relates to a method for inhibiting the proliferation of abnormal cells in a mammal which comprises applying directly to the situs of the abnormal cells an amount of a composition of this invention effective to inhibit said proliferation.

In another method of use aspect, this invention relates to a method of promoting the healing of a lesion in the tissue of a mammal which comprises applying thereto an amount of a composition according to this invention effective to promote the healing thereof.

In a further method of use aspect, this invention relates to a method of increasing the oxidative stability of a catecholic butane of Formula (II) which comprises mixing with said catecholic butane an oxidation inhibiting amount of ionic zinc.

In a still further method of use aspect, this invention relates to a method of enhancing the retention time of a catecholic butane at the situs of an affliction to which said catecholic butane is applied, which comprises applying said catecholic butane as a composition according to this invention containing an amount of said source of ionic zinc effective to enhance said retention time.

In a still further method of use aspect, this invention relates to a method for the treatment of disorders of the skin including acne, warts, inflammatory disorders and for alleviating bacterial, viral and fungal infections when applied to the situs of the disorder.

Detailed Description of the Invention

The term "source of ionic zinc" as used herein means a compound comprising ionic zinc in salt or chelated form, as opposed to metallic zinc. That source can be or can include the catecholic butane itself. When used herein, "zinc" means zinc in its ionic or divalent state and not metallic zinc.

The terms "afflicted situs" or "situs" as used herein refer to a localized area of pathology, infection, lesion or wound, or abnormal cells including solid tumors, and the immediately surrounding area.

The term "applying" as used herein embraces both topical applications to a surface of the situs and injection into the interior of the situs.

The term "mammal" as used herein includes feline, canine, equine, bovine, rodent and primate species, including cats, dogs, horses, rats, mice, monkeys and humans. Other animals, e.g., birds, can also be successfully treated with the compositions of this invention.

The term "abnormal cells" embraces both benign, premalignant and malignant cells. Examples of the former include the cells associated with adenomas, papillomas, etc. Examples of premalignant cells include actinic keratosis.

The term "proliferation" refers to the reproduction or multiplication and growth of cells.

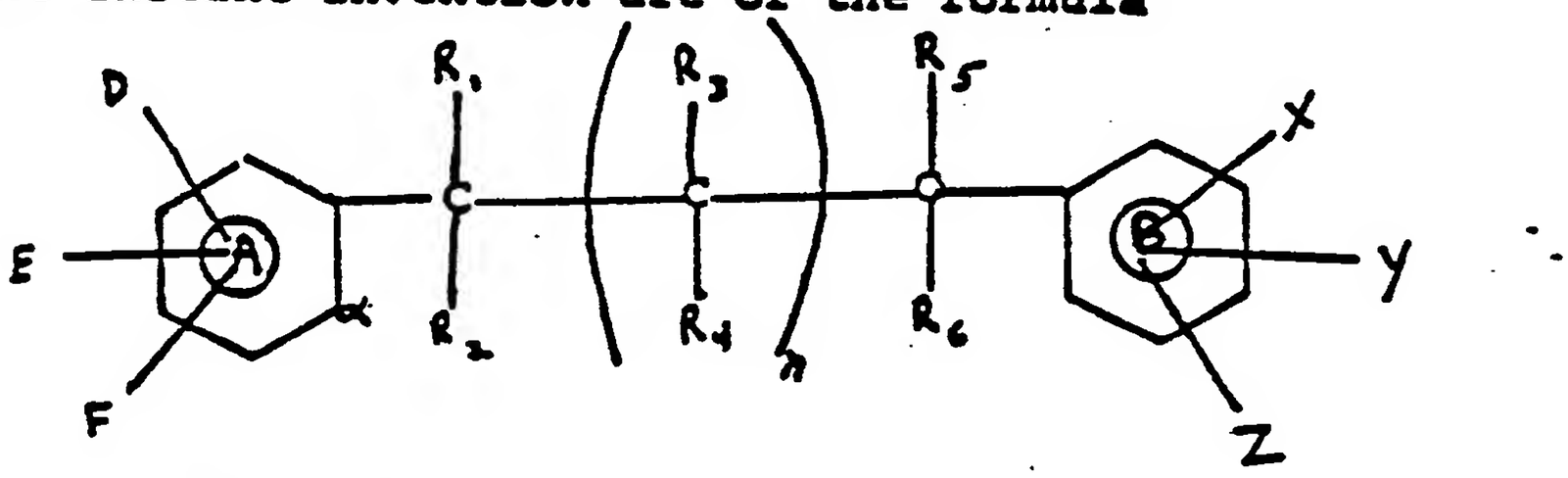
The term "escharotic" means a corrosive or caustic agent which is capable of killing healthy, living cells.

The term "nonescharotic concentration" means a concentration of the source of ionic zinc which does not kill living cells upon contact, e.g., as does zinc chloride when employed as an escharotic agent, e.g., at a concentration of about 40 weight percent or higher, depending on the delivery vehicle.

Compositions comprising a catecholic butane and zinc are particularly effective for the treatment of a variety of skin disorders and solid tumors. Improved results are obtained when the affected area is directly contacted with the instant compositions. With such compositions it has also been found that, surprisingly, the catecholic butane is retained by the tissue at the treatment site for a significant period of time before being distributed throughout the organism. This unexpected property of the instant compositions can increase the effectiveness of the treatment and also minimize any detrimental side effects of the components. Additionally, the combination of a catecholic butane and zinc allows a reduction in the concentration of each individual component while maintaining the efficacy of the composition. This reduction in the dosage level of the individual components obtained by combining the two active ingredients increases the safety of the composition. The instant compositions have been found to unexpectedly provide improved restoration of integrity to injured tissue. The presence of zinc has also been found to substantially increase the stability of the catecholic butane to oxidative reactions. The catecholic butane and zinc also unpredictably show no evidence of pharmacological antagonism.

The novel compositions of this invention are useful as antimicrobial, antifungal, antiviral and antitumor agents, as lesion healing promoting agents, e.g. for skin ulcers such as decubitus ulcers and lesions associated with osteomyelitis. They are useful in the treatment of keratoses, especially actinic keratosis including senile keratotic lesions. They are useful in treating a wide variety of premalignant and malignant skin tumors, basal cell carcinoma, squamous cell carcinoma and a diversified variety of melanotic lesions which are premalignant or malignant as well as certain cutaneous tumor manifestations of otherwise systemic diseases. The compositions have been found to be effective against solid tumors arising from all three embryonic tissue types, namely squamous cell carcinoma, e.g. lung carcinoma, arising from the ectodermal layer; adenocarcinomas, e.g. breast, renal and colon cancer, arising from the endodermal layer; and melanoma and brain cancers, arising from the mesodermal layer; and hematogenous tumors. The invention has also been found to be useful in the treatment of inflammatory disorders, acne and warts.

The catecholic butanes useful in the compositions of the instant invention are of the formula



wherein, D,E,F,X,Y,Z, may be H;OH; O-Alkyl or O-Acyl optionally substituted with hydroxy, alkoxy, substituted amino, carbalkoxy, or carboxy;

R_1-R_6 may be H; lower alkyl or lower alkoxyl optionally substituted with hydroxy, alkoxy, substituted amino, carboxyl, or carbalkoxyl; hydroxy; carbonyl; alkoxy; atyl; atalkyl;

n may be 0 to 5;

any of the aromatic rings in the molecule may contain up to 3 substituents from the following list: hydroxy; alkenoxy; alkyl, alkoxy or alkanoyl optionally substituted by hydroxy, alkoxy, substituted amino, carboxy, or carbalkoxy; CF_3; halo; carboxy; carbalkoxy; cyano; hydroxymethyl; sulfonic acid; sulfonamido; aminosulfonyl (i.e. - $NHSO_2R$); nitro; alkoxycarbonyloxy; aminocarbonyloxy; aroyloxy; aralkanoyloxy; heteroaroyloxy; glycosidyloxy; and

any two phenolic groups may be joined together by the following groups: CH_2, $\begin{matrix}CH_2-\\|\\CH_2-\end{matrix}$, HOP<(=O), Alkyl-O-P<(=O), R_2NP<(=O)

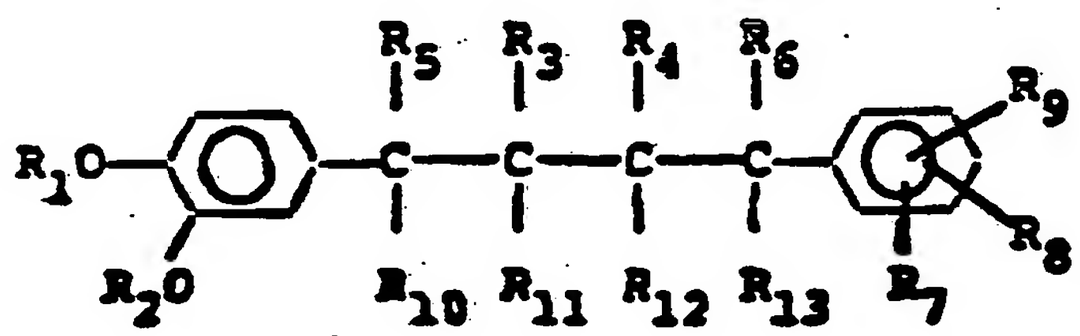
either of the rings A or B may be replaced by cyclohexyl, napthyl, tetrahydronapthyl, pyridyl, piperidinyl, quinolinyl, indanyl, indenyl;

any of the groups R_1 to R_6 may be joined together to form together with the other carbons to which they are attached, a 5, 6, or 7 membered ring optionally in - terrupted by an oxygen atom, or containing an oxygen atom and a carbonyl substituent, or containing a carbonyl substituent;

any of the groups R_3 to R_6 may be joined to ring A to form with it a 5, 6, or 7 membered ring;

any of the carbons in the chain between rings A and B, may be attached by a bond to the α position on ring A to form a 5, 6, or 7 membered ring.

The preferred catecholic butanes useful in the compositions of the instant invention are of the Formula



(II)

wherein R_1 and R_2 are independently H, lower alkyl

R_3, R_4, R_5 and R_6 are independently H or lower alkyl; R_7, R_8 and R_9 are independently H, hydroxy, lower

R_{10}, R_{11}, R_{12}, and R_{13} are independently H or lower alkyl.

Lower alkyl is intended to generally mean C_1-C_6 alkyl, and preferably R_3 and R_4 are C_1- C_3 alkyl. Lower acyl is intended to generally mean [C_1-C_6] acyl, with [C_2- C_6] being preferred. It will be appreciated by those skilled in this art that Formula II is directed to both the phenolic compounds and the conventional esters and ethers thereof.

Illustrative classes of compounds within the scope of Formula (II) are those wherein:

a) one or more of R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10}, R_{11}, R_{12}, and R_{13} are H, e.g., those wherein R_5 is H, R_5 and R_6 are H or R_5, R_6 and R_7 are H and R_8 and R_9 are OH or OR_1;

b) R_3 and R_4 each are CH_3 or C_2H_5 including those of a), especially those wherein R_5, R_6, and R_7 are H and/or R_8 and R_9 are OH and OR_1;

c) R_1 and R_2 are lower acyl, e.g., hydrocarbonacyl, preferably, alkanoyl, e.g., acetyl, propionyl, etc., including those of a) and b);

d) R_1 and R_2 are alike and R_8 and R_9 are OR_1 including those of a), b) and c); and

e) The compound is in the form of a single optical isomer or a mixture of such isomers, e.g., a racemic mixture or diastereoisomers including each of a), b), c) and d).

As used herein, lower alkyl represents, inter alia, methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, n-hexyl, and the like.

Lower acyl represents groups having the general formula RCO-, e.g., acetyl (CH_3CO-), propionyl (CH_3CH_2CO-), butyryl ($CH_3CH_2CH_2CO$-), and the like. When the catecholic butane compound is named as a substituted phenyl, the corresponding groups are acetoxy (CH_3CO_2-), propionyloxy ($CH_3CH_2CO_2$-), and butyroyloxy ($CH_3CH_2CH_2CO_2$-).

Examples of catecholic butanes include the d-, l-, racemic mixture of d- and l-, and meso-isomers of 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dihydroxyphenyl)butane; 1,4-bis(3,4-dimethoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-diethoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dipropoxyphenyl)-2,3-dimethylbutane; 1-(3,4-dihydroxyphenyl)-4-(3',4',5'-trihydroxyphenyl) butane; 1,4-bis(3,4-diacetoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dipropionyloxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dibutyroyloxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-divaleroyloxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dipivaloyloxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,3-dimethylbutyryloxylphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dihydroxyphenyl)-2-methylbutane; 1,4-bis(3,4-dihydroxyphenyl)-2-methyl-3-ethylbutane; and 1-(3,4-dihydroxyphenyl)-4-phenylbutane and 1-(3,4-dihydroxyphenyl-4-(2,5 dihydroxyphenyl) butane. It is contemplated that mixtures of these catecholic butanes can be used in the instant compositions.

The zinc may be present in the instant compositions as a cation, e.g., as a salt or a chelate of the catecholic butane itself or as pharmaceutically acceptable salt of another toxicologically acceptable anion, or as a mixture thereof. Pharmaceutically acceptable salts include those of inorganic acids, e.g., nitrate, sulfate, acetate, halides and phosphates, and those of organic acids, e.g., acetate, benzoate, citrate, caprylate, gluconate, etc., and mixtures thereof. Zinc chloride is especically preferred. As stated above, the term "zinc" as used herein means ionic zinc, rather than zinc metal.

While ionic zinc is preferred for use in the compositions according to the invention, it has been observed that other metals in the ionic state, particularly copper, vanadium and iron, have been found to be useful in the instant compositions. Other useful metals may include chromiun, yttrium, cobalt, platinum, cobalt, nickel, magnesium, aluminum, cadmium, antimony, mercury, rubidium and other rare earth metals. Generally, the highest oxidation state of a metal is preferred over lower oxidation states thereof.

The molar ratio of catecholic butane to zinc in the compositions of this invention can vary over a wide range. Ordinarily the molar ratio is between 100:1 and 1:100, more commonly between about 10:1 and 1:20. Preferably, the molar ratio ranges between about 5:1 and 1:15, and most preferably between about 3:1 and 1:10. The molar ratio preferably is such that at least one of the catecholic butane and zinc is present in the composition at a concentration effective to inhibit the proliferation of abnormal cells and the other of the two is present therein at a concentration effective to enhance that proliferation inhibiting activity, when the composition is applied in effective amounts to the situs of those cells. However, the molar ratio can be any that results in the composition exhibiting one or more of antifungal, antibacterial, antiviral and anti-tumor activity. In one aspect of this invention, the zinc is present at a concentration which prolongs the half-life of the catecholic butane at the situs of application. In another aspect, the zinc is present at a concentration which inhibits oxidation of the catecholic butane. In a

further aspect, the catecholic butane and zinc are present in concentrations to promote healing of a wound or lesion contacted therewith. The preferred molar ratio range depends upon the particular condition being treated as well as the method of delivery of the composition to the treatment site. The preferred range can be determined by normal pharmacological screening methods used in the art such as against the particular bacteria strain or strain of tumor cells. If desired, an excess of the zinc or the catecholic butane can be used as appropriate for the specific condition being treated.

For example, preferred molar ratios of the catecholic butanes to zinc with respect to two classes of tumors and exemplary application amounts/rate are shown in Table I.

TABLE I

Treatment/Use	Preferred Ratio of Catecholic Butane/Zinc	Exemplary Application Amount/Rate of Catechol/ Zinc Composition
Pre-Malignant Tumors	1:5-5:1 1-10% cat. but./ 15-1% zinc	Apply topically 2-150 mg/cm^2 of tumor. Repeat application when amount of prior application falls below about 5 mg/cm^2. Wound may be dressed until healing is complete. Healing period may extend for several months. Repeat daily as indicated by observation of tumor size reduction (i.e., if no reduction in size after 10 days, repeat 2-3 times daily; if reduction in size is served, after 10 days, repeat at daily intervals or sooner if reduction in size ceases to continue. Healing period may extend for several months. Alternatively, 0.1-20 ml. of composition may be injected intralesionally at the tumor site.
Solid Epithelial Tumors	1:15-5:1 1-10% cat. but./ 30-1% zinc	

The instant compositions can be applied topically to or injected into the treatment site, e.g., solid tumor, lesion or wound. When used for topical applications, the catecholic butane and the source of ionic zinc are usually formulated with a pharmaceutically-acceptable carrier. As used herein the term "pharmaceutically-acceptable carrier" refers to a material that is non-toxic, generally inert and does not adversely affect the functionality of the active ingredients. Carrier materials are well known in the pharmaceutical formulation art and include those materials referred to as diluents or vehicles. The carrier can be an inorganic or organic material and should have sufficient viscosity to allow spreading of the composition and provide good adherence to the membrane to which it is topically applied. Examples of such carriers include without limitation polyols such as glycerol, propylene glycol, polyethylene glycol, preferably of a molecular weight between about 400 and about 8000, suitable mixtures thereof, vegetable oils, etc. The viscosity of the formulation can be adjusted by methods well known in the art, for example by the use of a higher molecular weight polyethylene-glycol.

In addition to the catecholic butane, source of ionic zinc and carrier, the formulation can contain pharmacologically-acceptable additives or adjuvants such as antimicrobial agents, e.g. methyl, ethyl, propyl, and butyl esters of para-hydroxybenzoic acid, as well as

chlorobutanol, phenol, ascorbic acid, etc. The formulation can also contain thickening or gelling agents, emulsifiers, wetting agents, coloring agents, buffers, stabilizers and preservatives including antioxidants such as butylhydroxyanisole. The formulation can also contain penetration enhancers such as dimethyl sulfoxide, long-chain alcohols such as nonoxynol, long-chain carboxylic acids, propylene glycol, N-(2-hydroxyethyl)pyrrolidone, 1-dodecyl-azacycloheptan-2-one, and the like. Depending on the method of application and the disease being treated, it may be desirable to use absorption-delaying agents such as aluminum monostearate and gelatin.

The composition of the formulation can be adjusted using components well-known in the formulation art to provide a pharmaceutical formulation which is a gel, cream, ointment, solid, liquid, semi-solid, etc. The particular physical form of the formulation depends on the desired method of treatment and the patient to be treated.

Typical formulations of the pharmaceutical compositions of this invention are set forth in Table II:

TABLE II

Application Form	Formulation	(Per 100 mgs.)	
Ointment	Zinc chloride	10.0	(preferred range: about 0.05-35)
	Catecholic butane	5.0	(preferred range: about 0.1-30)
	Peg 400	4.2	
	Peg 8000	51.7	
	Water	19.0	
	Ascorbic acid	0.1	
Gel	Zinc chloride	10.0	(preferred range: about 0.05-35)
	Catecholic butane	5.0	(preferred range: about 0.1-30)
	Standard denatured alcohol	10.0	
	Propylene glycol	22.5	
	Water	43.4	
	Non-ionic surfactant	6.0	
	Xantham gum	3.0	
	Ascorbic acid	0.1	

TABLE II (CONTINUED)

Cream	Zinc chloride	10.0	(preferred range: about 0.05-35)
	Catecholic butane	5.0	(preferred range: about 0.1-30)
	Ascorbic acid	0.1	
	Benzyl alcohol	5.0	
	Propylene glycol	23.0	
	Water	25.4	
	Stearyl alcohol	7.0	
	Cetyl alcohol	4.5	
	White petrolatum	13.0	
	Poloxyl-40 stearate	7.00	
Solid	Zinc chloride	5.00	(preferred range: 0.05-35)
	Catecholic butane	5.00	(preferred range: 0.1-30)
	Carnuba wax	8.88	
	Beeswax	13.32	
	Lanolin anhydrous	4.44	
	Cetyl alcohol	4.44	
	Ascorbic acid	0.10	
	Castor oil	57.70	
	Water	1.20	
Injectible Liquid	Zinc sulfate.$7H_2O$	2.00	(preferred range: 0.05-35)
	Catecholic butane	1.05	(preferred range: 0.1-30)
	Water	33.94	
	Glycerine	36.44	
	Glycine	1.52	
	Sodium ascorbate	0.05	
	Propylene glycol	25.00	

For administration by injection, the composition is formulated as a solution or suspension having a low enough viscosity to be injected. The composition suitable for injectable use must be sterile and fluid to the extent that easy syringe injection exists. It should also be stable under conditions of manufacture and storage and be preserved against contamination by microorganisms. Additionally, the pH of the composition must be within a range which does not result in tissue damage.

The concentrations of the catecholic butane and the ionic zinc in a particular formulation depend on the condition being treated, the method of application, i.e. topical or injection, the rate of delivery of the active ingredient(s) to the treatment site, and the number of applications of the formulation which can be used. Additionally, certain catecholic butane compounds are more effective in treating particular conditions than are

other analogs. The optimum amount of a specific catecholic butane for treating a condition cannot be predicted at this time. However, an effective range can readily be determined by procedures known to those skilled in the art and explained elsewhere herein. It has been found that ordinarily a lower concentration of catecholic butane and ionic zinc can be used when treating a microbial infection than when treating a solid tumor. The concentration of ionic zinc in the formulation can likewise depend upon the condition being treated and the particular catecholic butane or combination of butanes being used. As discussed hereinabove, it may be desirable to have a substantial excess of one component, for example ionic zinc, present in the formulation in order to effectively treat the particular condition.

In practice, it is preferred that a formulation contain the lowest concentrations of catecholic butane and ionic zinc which effectively treat the condition with the desired number of applications, i.e. a lower effective dose rate can be tolerated if multiple applications are used. This low concentration limit is dependent upon the delivery effectiveness of the carrier vehicle. Preferably, the catecholic butane and zinc together comprise between about 0.5 and about 80 weight percent of the formulation. Recognizing that it may be possible to use lower concentrations depending on the delivery of the carrier, it is expected that a formulation for treating microorganisms or fungi would ordinarily contain between about 0.001 and about 20 weight percent of catecholic butane and between about 0.001 and about 30 weight percent zinc. In the treatment of solid tumors, It is ordinarily expected that the formulation contain between about 0.1 and about 30 weight percent catecholic butane and between about 0.05 and about 35 weight percent zinc. Preferably at least one of the catecholic butane and the zinc is present in the formulation at a concentration of

at least about 0.5 weight percent, more preferably at least about 1.0 weight percent. As used herein, the weight percent in the formulations refer to the concentrations of materials being effectively delivered to the treatment site. As stated above, it is contemplated that formulations can be prepared that have significantly higher concentrations of catecholic butanes and zinc depending upon the carrier and additives being used. If the carrier substantially retains the catecholic butane and zinc or releases them at a slow rate, the concentrations of these materials in the formulation can be substantially increased and in fact may have to be substantially increased in order to provide an effective treatment. The concentrations of active ingredients in a particular formulation required to provide a particular effective dose (ED) can be generally determined by a person skilled in the pharmaceutical formulation art based upon the properties of a carrier and the particular additives introduced into the formulation. It is also expected that a formulation which is being applied topically can contain a higher concentration of catecholic butane and zinc than a composition being injected, for example into a solid tumor.

A preferred embodiment of the instant invention comprises compositions containing nordihydroguaiaretic acid, i.e. 1,4-bis(3,4-dihydroxylphenyl)-2,3-dimethylbutane, and zinc chloride. This combination has been found to be particularly effective in treating acne and Propionibacterium acnes, decubitus ulcers, osteomyelitis, actinic keratosis and solid tumors. Since zinc chloride at high concentrations is an escharotic material, it is preferred that the concentration of zinc chloride delivered to the treatment site be maintained below a concentration which is escharotic to the healthy tissue. Although the effective concentration of zinc chloride as well as nordihydroguaiaretic acid delivered to the treat-

ment site depends upon the carrier and other additives included in the formulation, ordinarily the concentration of nordihydroguaiaretic acid in the formulation will range from about 0.01 to about 40 weight percent and the concentration of zinc chloride in the formulation will range from about 0.01 to about 35 weight percent. These ranges are provided by way of description and not by way of limitation since it is recognized that the concentration can be adjusted over a wide range depending on the carrier material, number of applications used, etc., as described hereinabove.

The instant compositions have the advantage of the beneficial and unexpected interaction between the catecholic butane and ionic zinc. This beneficial relationship is not understood at this time; but it allows the concentrations of the catecholic butane and zinc to be reduced to lower, more toxicologically acceptable levels while obtaining comparable or superior results to the use of higher concentrations of individual components. Thus, the concentration of zinc chloride can be reduced to below an escharotic level in the formulation.

The pH of the formulation can be important in assuring stability of the catecholic butane as well as assuring that the formulation is physiologically acceptable to the patient. Many of the catechols, particularly nordihydroguaiaretic acid, are susceptible to oxidation, for example by air. Such oxidation can result in discoloration of the formulation rendering it unacceptable for pharmaceutical use. These catechols are more stable against oxidation at lower pH levels. Therefore, it is preferred that if the formulation is to be exposed to oxidizing conditions the pH be maintained below about 7 and preferably below about 6 in order to provide maximum stability for the catechol against oxidation. However, if oxidizing conditions can be avoided, for example by storage of the formulation under an inert atmosphere such

as nitrogen, a higher pH can be used. The pH of the formulation can be maintained through the use of toxicologically-acceptable buffers. Such buffers are well known in the pharmaceutically formulation art.

It has been found that the presence of ionic zinc in a catecholic butane formulation can substantially retard the rate of oxidation of the catechol, i.e. increase the stability of the catecholic butane to oxidation. This has significant advantages in that the introduction of unknown oxidation products of the catecholic butanes is minimized and the shelf-life of the catecholic butane compositions is increased. While not intending to be bound by a possible explanation of this not fully understood phenomena, experimental evidence set forth in the instant examples indicates that the ionic zinc serves to stabilize the semiquinone free radical and radical-anion intermediates formed during the oxidation process possibly by forming a complex with the catechol. Surprisingly, zinc ions dramatically decreased the decay rates of these radicals compared to other metal ions tested. Consequently, the stability to oxidation of a catecholic butane formulation such as one containing nordihydroguaiaretic acid can be increased by the addition of zinc ions in the form as discussed hereinabove. It is expected that the presence of ionic zinc in a molar ratio zinc to catecholic butane of about 1:50 can increase the stability of the catechol; however, it is preferred that the molar ratio zinc to catechol be at least about 1:5, and most preferably at least about 1:2 with an excess of zinc contemplated as being most beneficial.

The compositions of the instant invention have also been found to be useful in the treatment of lesions, draining lesions, and draining wounds which show impaired healing. As used herein the term "lesion" refers to any pathological or traumatic discontinuity of tissue. A "wound" is a lesion which results from a bodily injury

caused by physical means. Lesions which do not readily heal can be manifestations of conditions, diseases or infections, for example, cutaneous ulcers, osteomyletis, acne vulgaris, draining fistulas, etc. Not uncommonly, lesions do not heal properly and continue to drain which results in discomfort to the patient and a continued threat of severe infection. Such conditions in which tissue does not readily grow to heal the lesion or wound can be the result of bacterial infection or other causes not fully understood. Exposed areas, created by the sloughing off of necrotic matter, generally result in pus formation (suppuration). Although the exact mechanism is unknown, direct contact of the exposed area of the lesion with the instant compositions has been found in clinical studies to substantially aid the healing process, possibly by inducing the formation of granulation tissue. The instant compositions are beneficial in promoting healing of lesions in patients having serum zinc levels within the range generally accepted as being normal. This promotion of healing has significant advantages, for example, in the treatment of solid tumors directly or the situs from which such tumors have been surgically removed in that healing is promoted concurrently with inhibiting the proliferation of any tumor cells which might remain at the site of surgery.

In topical applications the instant compositions are applied to the affected area or afflicted situs of the patient. The term "topical" refers herein to the surface of the epidermal tissue, especially the skin, the surface of tumors on the skin which have been debrided or otherwise modified, as well as sites from which solid tumors have been removed either from the skin or internally. The instant compositions can be particularly useful in conjunction with surgery for removal of internal cancers to eradicate residual tumor cells and act as a prophylactic against local recurrence and metastatic

spread of the tumor. The instant compositions can be used instead of surgery when there are cosmetic considerations due to the normally improved appearance of healed situs treated with the instant compositions compared to surgery alone.

Application by injection can be used for treatment of solid tumors in which removal by surgery is not desired or for which surgery is not medically advisable. In this procedure the instant composition is injected directly into the tumorous growth. The injection may be accomplished at a number of sites in the growth in order to provide the maximum contact between the instant composition and the tumorous cells.

As used herein the term "solid tumor" refers to tumors in which a plurality of tumor cells are associated with one another, i.e. contiguous and localized within a confined site. This is to be contrasted with "fluid" or "hematogenous" tumors in which the tumor cells occur primarily as unassociated or individual cells, e.g. leukemia. Solid tumors generally propagate on host tissues such as the epithelial, the connective and supportive tissues as well as other tissues located throughout the body. Examples of epithelial tumors include papillomas and carcinomas such as squamous cell carcinoma, basal cell carcinoma, adenoma, adenocarcinoma, cystadenoma and cystadenocarcinoma. Examples of supportive and connective tissue tumors include sarcomas and their benign counterparts such as fibrosarcoma, fibroma, liposarcoma, lipoma, chondrosarcoma, chondroma, leiomyosarcoma and leiomyoma. Examples of other tissue tumors include gliomas (brain tumors) and malignant melanomas.

The compositions of the instant invention have been found to be particularly effective against the following solid mammalian tumors: human tumors including malignant melanoma, squamous cell carcinoma, lung squamous cell carcinoma, breast adenocarcinoma, glioma, glioastrocy-

toma, renal-cell carcinoma, colon, and basal cell epithelioma; canine tumors including mast cell carcinoma, squamous cell carcinoma, mammary adenoma, breast adenocarcinoma, perianal adenocarcinoma, perianal adenoma, sebaceous adenoma, and basal cell carcinoma; and equine tumors including papilloma, malignant melanoma, sarcoid and squamous cell carcinoma.

In order to determine the efficacy of a composition as an antimicrobial, antiviral, antifungal or antitumor agent, the composition is commonly initially tested by in vitro screening methods. When tested against microorganisms, the composition is commonly applied to a colony at different concentrations and the kill ratio determined. In the treatment of tumors, initial screening is commonly done by the human tumor clonogenic assay. It has been reported that clinical correlations from retrospective analysis and prospective clinical trials with such clonogenic assays have indicated that there is a 60 to 70 percent correlation between in vitro sensitivity and clinical response. The studies have also indicated that there is a greater than 90 percent correspondence between in vitro resistance and treatment failure. However, the screening of new antitumor agents is still primarily being conducted using a variety of tumor models in vivo. The National Cancer Institute is currently using in vivo tumor models which include the L-1210 lymphocytic leukemia, B-16 melanoma, M-5076 carcinoma, 3 transplantable murine tumors, and the MX-1 human mammary tumor xenograph.

In preparing a formulation suitable for topical application, the catecholic butane is normally mixed with a suitable solvent. Examples of solvents which are effective for this purpose include ethanol, acetone, acetic acid, aqueous alkaline solutions, dimethyl sulfoxide, glycerine, glycerol, propylene glycol, suitably high boiling ethers, nonoxynol, polyethylene glycol, etc.

The zinc ions, commonly in the form of a toxicologically-acceptable salt, are mixed with a suitable solvent such as water or polyethylene glycol of low molecular weight, e.g. 200-400. The ionic zinc can be added in the form of readily available salts such as acetates or other aliphatic acid salts while the preferred anion, e.g. chloride, can be added in the form of its readily available salts such as sodium chloride. In the event there is not complete solubilization, the mixture can be milled to obtain a fine suspension.

The catecholic butane composition and the source of ionic zinc are mixed in appropriate amounts to achieve the desired concentrations. Additives, adjuvants, other carriers, etc., can be introduced at any stage of the preparation as appropriate. When the formation of a metal chelate or complex is desirable, the ordering of mixing of ingredients and the pH of the formulation can be critical. When chelates or complexes are desired, compounds which can serve as counter-ligands are preferably provided so that discreet "molecular" entities are formed rather than polymers of indeterminant length. Such counter-ligands include ethylenediamine tetraacetic acid (EDTA), ethylenediamine diacetic acid (EDDA), ethylenediamine, ammonia, ethanolamine, amino acids, etc.

While it has been observed that zinc and other metal salts act to enhance the ability of a given amount of the compounds according to the invention to reduce or eradicate tumors or retard tumor growth over and above the ability of the same amount of the compound alone to

reduce tumor size, it has also been surprisingly observed that effective concentrations of the compounds according to the invention without the presence of metal ions, e.g., zinc ions, can be administered to mammals to achieve a similar effect. Similarly, the compounds according to the invention have been found to be effective and non-toxic for the other treatments and diseases disclosed herein.

In this regard, the concentration of the compounds according to the invention in a particular formulation depends on the particular neoplasm or disease being treated, the method of application, i.e., topical or injection, and the rate of delivery of the active ingredient(s) to the treatment site. It has been found that it is necessary to contact the tumor cells with at least a threshold amount of NDGA to observe an inhibition in growth of the neoplasm. However, an effective range can readily be determined by procedures known to those skilled in the art and explained elsewhere herein.

The following examples are included by way of illustration and not by way of limitation. Unless otherwise indicated, the nordihydroguaiaretic acid used in the instant Examples was the meso-isomer and is designated NDGA. Other isomers are indicated, e.g., d,l-NDGA.

EXAMPLE I

The catecholic butane 1-(3,4-dihydroxyphenyl)-4-(2,3,4 -trihydroxyphenyl) butane was prepared by the following procedure.

500 grams of 3,4-dimethoxydihydrocinnamic acid was suspended in 1.6 liters of methanol containing 250 ml of 2,2-dimethoxypropane. To this mixture was added

dropwise a solution made by adding 20 ml. of acetyl chloride to 400 ml of methanol. The resulting mixture was stirred overnight at room temperature and finally at reflux for one hour. The solvent was evaporated to give a syrup in quantitative yield, 533 g.

To 912 ml. of lithium aluminum hydride (1M in THF) was added dropwise 213 g. of 3,4-dimethoxydihydrocinnamic acid methyl ester dissolved in 900 ml of dry THF at such a rate as to maintain gentle reflux (5 hours). The reaction mixture was stirred overnight at room temperature, cooled in an ice bath and treated dropwise with ammonium chloride solution (saturated) (104 ml) over a two hour period. After stirring for several hours, the reaction mixture was diluted with 500 ml. of THF, filtered and the filtrate evaporated in a vacuum to give 160 g. (86%) of a light yellow oil.

3-(3,4-dimethoxyphenyl) propanol (202 g) was added to 218 ml of triethylamine in one and half liters of methylene chloride. This solution was cooled to -10°C in an ice salt bath and 87.6 ml. of methanesulfonyl chloride was added dropwise over a one and a half hour period while stirring rapidly. Stirring was continued for another hour and the mixture was washed with 700 ml. of ice water, 700 ml. of 3N hydrochloric acid, 700 ml. of saturated sodium bicarbonate and finally with 700 ml. of brine. The organic phase was dried with sodium sulfate and evaporated in a vacuum to give an orange oil in quantitative yield, 282 g.

3-(3,4-dimethoxyphenyl) propanol methanesulfonate, 282 g., (1.029 mol.); KBr, 282 g. (2.37 mol.) and dicyclohexano-18-crown-6, 19.2 g. (0.01515 mol.) were stirred in refluxing acetonitrile, 2.8 liters (dried over 3A molecular sieves) for 22 hours. The mixture was filtered and the filtrate evaporated in a vacuum to give

an orange oil, 267 g. The product could be purified by vacuum distillation at 0.5 mm Hg, b.p.=113-116°C.

3-(3,4-Dimethoxyphenyl) propyl bromide, 25.9 g., in 50 ml. of dry tetrahydrofuran (dried distillation from LAH) was placed in a dropping funnel. Magnesium powder, 2.5 g., and a trace of iodine was placed in a dry three neck flask with nitrogen inlet and reflux condenser. The reaction started upon addition of the liquid reactant and reflux was continued over a three hour period during which time the metal dissolved in the stirred solution. The reaction was cooled and the volume made up to 200 ml. to form a 0.5M solution in dry THF.

2,3,4-Trimethoxybenzaldehyde, 1.96 g. (0.01 mole), dissolved in 20 ml. of dry THF and 20 ml. of the 0.5M Grignard reagent from 3-(3,4-dimethoxyphenyl)propyl bromide in THF was added dropwise at ice temperature. The mixture sat over night at room temperature. The solution was evaporated in a vacuum and 20 ml. of ethanol was added carefully followed by excess sodium borohydride. Refluxing for a few minutes destroyed the yellow color of the small amounts of ketone and other unsaturated impurities formed from oxidation of the product. Most of the ethanol was evaporated and the residue partitioned between water and ether, 50 ml. of each. The ether phase was dried over sodium sulfate and evaporated to give 4.65 g. of a pale yellow oil.

The 4-(3,4-dimethoxyphenyl)-1-(2,3,4-trimethoxyphenyl) butanol, 3.65 g., was treated with excess sodium hydride, I g., and methyl iodide, one ml, in 25 ml. of dry dimethylformamide during one hour of stirring. Water was added carefully dropwise at first and finally 500 ml. of water was added. The product was extracted three times with 50 ml. of chloroform and the solvent evaporated to give a colorless crude oily product that

can be used in the next step without further purification.

About 100 ml of anhydrous ammonia was condensed into a three necked flask with a dry ice condenser and dry ice bath. The flask was protected from moisture with a soda-lime tube and flow of dry nitrogen. One gram of clean sodium metal was dissolved in the liquid ammonia and the whole of the crude product in 20 ml of dry tetrahydrofuran was added as quickly as possible. The dark blue solution was stirred rapidly for twelve minutes before enough methanol was added to destroy the blue color. Evaporation of the solvent under a vacuum gave a thick residue to which 500 ml. of water was added. The water solution was extracted twice with 50 ml. of chloroform that left three grams of oily residue on evaporation. Chromatography of this crude product on 300 g. of silica-gel using chloroform as an eluate gave 2.3 of pure 1-(3,4-dimethoxyphenyl)-4-(2,3,4-trimethoxyphenyl) butane (one spot on TLC).

A 1.15 g. sample of 1-(3,4-dimethoxyphenyl)-4-(2,3, 4-trimethoxyphenyl) butane was refluxed for nine hours in 50 ml. of 48% hydrobromic acid under an inert nitrogen atmosphere. Standing over the weekend allowed 641 mg. of tan product to settle out in the freezer. This material was recrystallized under inert atmosphere from methanol-water 1:20 to give light pink crystals, m.p.=165-167°C.

The following compounds were prepared by a similar procedure:

a) 1-(3,4-Dihydroxyphenyl)-4-(3,4,5-trihydroxyphenyl)butane;

b) 1-(3,4-Dihydroxyphenyl)-4-phenylbutane

[illegible] SHEET

c) 1-(3,4-Dihydroxyphenyl-4-(2,5-dihydroxyphenyl) butane;

d) 1,4-Di(3,4-dihydroxphenyl)-1,2,3,4-tetramethylbutane

e) 1,4-Di(3,4-dihydroxyphenyl)-2-methyl-3-ethylbutane

f) 1,4-Di(3,4-dihydroxyphenyl)-1-propyl-2-methyl-3-ethylbutane

EXAMPLE 2

To liquid ammonia (approximately 150 ml.) was added powdered ferric chloride, 150 mg., then small pieces of sodium, 1.53 g., were added and the blue color was allowed to dissipate over about a 20 minute period. To the resulting grey suspension of sodamide was added solid 3,4-dimethoxypropiophenone, 11.64 g., in small portions and the mixture was stirred for about five minutes. Solid alpha bromo-3,4-dimethoxy-propiophenone, 16.38 g., was then added in small portions to the grey-green mixture. After the mixture stirred for one hour 8. g. of ammonium chloride and 150 ml. of dichloromethane was added. The ammonia was allowed to evaporate and the mixture filtered while the solid residue was extracted twice with additional dichloromethane. Evaporation of the solvent to a small volume and dilution with methanol allowed crystallization of 19.75 g. of product.

The 2,3-bis(3,4-dimethoxybenzoyl)butane, 1.65 g., was not soluble in toluene, 25. ml, so enough dry tetrahydrofuran, 25. ml, was added to make the solution complete. An excess, 5. ml, of sodium dihydrobis(2-methoxyethoxy)aluminate (Vitride$_R$ 70% in toluene) was added and stirred at room temperature for several days. A saturated solution of sodium sulfate was added and the

solid filtered off and washed with ether. Evaporation of the combined filtrates gave a thick colorless oil that still contained some solvent. Crystallization from 20. ml of methanol/water gave 1.15 g. of colorless product.

To one gram of lithium aluminum hydride in 100 ml of dry tetrahydrofuran at ice temperature was added dropwise 3.86 g. of 2,3-bis(3,4-dimethoxygenzoyl)butane dissolved in 30. ml of dry tetrahydrofuran during rapid stirring under nitrogen. The mixture was allowed to slowly warm to room temperature and finally refluxed for one hour. One ml of saturated sodium sulfate was added and the solids removed by filtration and washed with tetrahydrofuran. Evaporation of the solvent in a vacuum gave a colorless oil that crystallized on the addition of ether to give 3.93 g of product.

1,4-(3,4-dimethoxyphenyl)-2,3-dimethylbutane-1,4-diol,3.9g, was dissolved in dry dimethylformamide, 50.ml, and one half gram of sodium hydride was added in small portions to the rapidly stirring and externally cooled solution. Methyl iodide was added dropwise as the solution continued to stir under a nitrogen atmosphere.

After one hour at room temperature, water was added dropwise carefully at first and then several hundred ml was used to dilute the reaction mixture. The mixture was extracted with chloroform three times, backwashed with water three times, dried over sodium sulfate and evaporated to give a yellow oil, 4.0 g., that could be crystallized from ether to give 1.6 g. of colorless solid.

The 1,4-(3,4-dimethoxyphenyl)-1,4-dimethoxy-2,3-dimethyl- butane, 1.6 g., dissolved in 20. ml of dry THF

was added to excess sodium, 200 mg., dissolved in 200 ml of liquid ammonia and stirred for twelve minutes. The excess sodium was destroyed with the addition of three ml of ethanol. The ammonia was allowed to evaporate and a vacuum was used to evaporate most of the THF. Water was added and the mixture was extracted with chloroform. Evaporation of the chloroform gave 1.4 g. of colorless oil, d,l-NDGA tetramethyl ether (racemic). TLC on silica-gel using chloroform as a solvent gave one spot RF = 0.4. The 1H- and 13C-NMR was taken and compared with NDGA tetramethyl ether (meso).

The tetramethyl ether 100 mg., was placed in a tube containing one ml of 46% hydrobromic acid and the tube cooled in liquid nitrogen and sealed in a vacuum. The tube was allowed to warm and then placed in a bath of refluxing hydrobromic acid for nine hours. Water was added to the opened tube and cooling gave 82.6 mg. of darkcolored crude product that was purified via silica-gel chromatography using ether as the solvent. Crystallization from ether/hexane and methanol/water gave colorless product.

Other ethers are synthesized in a similar fashion starting with the appropriate etherified propriophenones. They may also, be prepared by refluxing the corresponding 1,4-phenolic butanes with the appropriate alkyl halide in acetone, with stirring and with potassium carbonate as the acid acceptor.

Nordihydroguaiaretic acid, Tetra Pivaloate

Nordihydroguaiaretic acid, 1.0 g., was dissolved in 10. ml. of dry pyridine and 10. ml of xylene in a dry nitrogen atmosphere and trimethylacetyl chloride, 10. ml added while stirring. The mixture was refluxed for four hours. The solvent was evaporated in a vacuum to an

oil, washed with water and extracted into methylene chloride. The methylene chloride was dried over sodium sulfate and evaporated to give an oil, 2.1 g. that solidified on standing.

Nordihydroguaiaretic acid, Tetropropionate

Nordihydroguaiaretic acid, 3.g, in 50 ml. of pyridine was cooled in ice and propionyl chloride, 5.g., was added dropwise (excess). The mixture was stirred overnight, poured into ice water and extracted with ether. The ether was evaporated and the solid residue was crystallized from alcohol-water to give an almost quantitative yield of colorless crystals.

EXAMPLE 3

A number of experiments were performed to determine the antitumor activity of the compositions according to the invention against B-16 melanoma and Sarcoma-180 solid tumor growth in mice.

The test compounds comprised varying compositions of nordihydroguaiaretic acid (NDGA), zinc chloride and excipients formulated into a polyethylene glycol (Pego) base to obtain an appropriate consistency for application. Some of the compositions included an additional compound, quercetin. The wt. ratio of zinc chloride to NDGA generally ranged from about 4:1 to 1.5:1.

Both types of the tumors were grown intradermally or subcutaneously in the mice. When adequate tumor size was achieved, the mice were divided into control and test groups. The tumors were punctured uniformly and then either a test compound or a control by topical application was applied to the surface of the tumor. In

some cases, the tumors were injected with the test compound or the control.

Almost all of the tumors demonstrated a significant reduction in size or were completely eliminated by the test compounds containing zinc chloride and NDGA.

Exemplary compositions of the mixtures are given in Table 2:

Table 3

Mixture	$ZnCl_2$	NDGA	EDTA	H_2O	PEGO
53	27.5	6.9	14.7	18.3	32.6
54	28	6.8*	14.7	18.2	32.9
55	16.4	6.9	8.6	18.0	32.2

* d,l NDGA

These mixtures were tested for their potential antitumor activities against B-16 melamonas grown in mice in accordance with the procedure discussed above. The results are given in Table 2a.

Table 3a

Mixture	n	T/C	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
53	10	0	0 (575±270)	80 (0)	80 (60)
53	10	8	51±118 (711±286)	70 (0)	100 (100)
54	10	0	0 (711±286)	60 (0)	100 (100)
55	10	73	522±356 (711±286)	10 (0)	100 (100)

EXAMPLE 4

Fifteen older dogs having perianal adenomas were treated topically with the NDGA plus zinc salt ointment having a strength of 55% (w/w).

To 36.7 grams of powdered Larrea divaricata extract, containing 85% of weight NDGA, were added 24.5 grams of powdered rosehips and the mixture was mixed in a blender for 5 minutes. The blended mixture was then mixed with 100 milliliters of an aqueous solution containing 185.9 grams zinc chloride to form a paste. The paste was allowed to stand at room temperature for 24 hours. Thereafter, it was stirred and then placed in a screw-capped glass container. The container was placed in a humidified oven at 40°C for 5 days. This incubated paste was then suspended in 500 milliliters of water and shaken at room temperature for 24 hours on a reciprocating shaker. The zinc chloride extract

solution was then evaporated to near dryness on a rotary evaporator at 90°C under reduced pressure. A sufficient quantity of this dried zinc chloride extract was added to 120 grams of an ointment base consisting of 10% (w/w) stearyl alcohol and 90% (w/w) polyethylene glycol to obtain an ointment containing 70% (w/w) of the extract.

The normal treatment for such a condition is surgery; however, these older dogs were poor surgical risks. The tumor of each dog was biopsied and the ointment was applied topically into the biopsied incision. The duration of treatment varied depending upon the severity of the adenoma. Dogs with simple circumscribed adenomas required only one treatment. The dogs with more advanced adenomas generally required more than one treatment which were given three to five days apart. The treatment was successful in thirteen of the fifteen dogs. The treatment was not successful in two of the dogs which had extremely advanced cases of perianal adenomas.

EXAMPLE 5

Test compositions were prepared according to the following general method to test the activity of the compositions according to the invention against human breast adenocarcinoma, MX-1.

The NDGA, BHT (butylated hydroxytoluene), and Pego 400 were measured and mixed together with heating until melted and dissolved. Pego Base (50% Pego 400, 45% Pego 3350 and 5% stearyl alcohol) was prepared by mixing and heating the components together in a separate container until they dissolved. $ZnCl_2$ and EDTA were dissolved in water with heating and stirring in a separate container. The ingredients in each of the separate containers were added together in amounts needed to give the

cancer (glioma); melanoma; and colon cancer, CX-1. The test composition with the approximate wt/wt percentages given below was prepared according to the procedure previously described in Example 4. The control composition was Pego 400.

Ingredient	Test Composition 1	Control
BHT	0.16	-
EDTA	2.10	-
NDGA	0.66	-
$ZnCl_2$	4.26	-
H_2O	2.62	-
Pego Base	1.43	-
Pego 400	88.77	100

The composition was then tested for its effect on human tumors of varying origin implanted in athymic mice as previously described. Generally, there were ten mice in each group tested with Pego 400 control. Instances in which the number of mice varied are specifically indicated.

Results are given in Table 7.

TABLE 7

Tumor Type	Test Composition	Tumor Free at 60 Days	Premature Death	Tumor At Death	Recurrence
LX-1 (lung)	1	8	0	2	0
	control	0	0	5	0
MX-1 (breast)	1	8	0	2	1
	control	0	0	2	0
RX-1 (Renal)	1	8	1	1	0
	control	0	1	5	0
Glioma (Brain)	1	6	0	0	0
	control	0	0	2	0
Melanoma	1	10	0	0	0
	control	0	0	5	0
CX-1 (Colon)	1	8	1	2	0
	control	0	0	5	0

EXAMPLE 8

A number of catecholic butane compositions were formulated into test compositions according to the following general method, and tested for activity against human breast adenocarcinoma, MX-1.

Zinc chloride was dissolved in Pego 400 to prepare a stock solution. The amount of organic compound required to give the final concentration given below was separately dissolved in Pego 400.

The two solutions were mixed to give a final concentration in each test composition of zinc chloride at 0.69 wt/wt % and each organic compound at a molar concentration equivalent to 4.4 wt/wt % of NDGA.

The test compositions in Table 7 were tested for their effectiveness as antitumor agents against xenografts of the human breast adenocarcinoma, MX-1, grown in athymic mice. They were administered to five animals by intratumor injection. Animals were administered 0.05 ml of test composition unless indicated otherwise.

TABLE 8

Animal Organic Compounds	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
Pego control	0	0	5	0
NDGA	4	1	0	0
d,l NDGA	5	0	0	0
NDGA Tetracetate	4	0	1	0
NDGA Tetrapropionate	4	0	1	1
1,4-bis(3' -methoxy-4' -hydroxyphenyl Butane	2	0	3	0
1,4-bis(3' -methoxy-4' -hydroxyphenyl)-2, 3-dimethyl butane	4	0	1	0
1-(3',4'-dihydroxyphenyl) -4-(2',3',4'-trihydroxy-phenyl)-butane	2	1	3	0
1-(3',4'-dihydroxyphenyl) -4(3',4',5'-trihydroxy-phenyl)-butane	3	0	2	1
1-(3',4',-dihydroxyphenyl) -4-(2',5'-dihydroxyphenyl) -butane	5	0	0	0
1-(3',4'-dihydroxyphenyl) -4-phenyl butane	3	1	1	0
1-(3',4'-dihydroxphenyl) -4-(2',4'-dihydroxyphenyl) -butane	2	0	3	3

EXAMPLE 9

Various zinc salts were tested in combination with NDGA to determine the effectiveness of the compositions according to the invention against xenografts of human breast adenocarcinoma, MX-1, grown in groups of five athymic mice.

The tumors were implanted subcutaneously in the left flank of the mice and the tumors were allowed to grow until they reached an approximate size of between 25 and 100 mm^2 (length x width). The mice were given a single 0.010 ml intratumor injection of the test composition. The concentration of the various metal salts in the test compositions was 0.73% (wt/wt) metal salt and 1.0% (wt/wt) NDGA, in a PEGO 400 base. The results of these test compositions are summarized in Table 8.

TABLE 9

Test Compound	Tumor Free at 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
$ZnCl_2$	4	0	3	3
$ZnSO_4 \cdot 7H_2O$	2	0	3	3
$ZnBr_2$	2	0	3	3
Zn Acetate $\cdot 2H_2O$	2	0	3	3
$Zn(NO_3)_2 \cdot 6H_2O$	3	1	1	1
$ZnCl_2$ (without NDGA)	1	0	19	19

In a separate trial, solubilized zinc gluconate demonstrated efficacious results in the <u>in vitro</u> inhibition of clonogenic human lung tumor cells (LX-T) when combined with NDGA.

EXAMPLE 10

This example describes the antineoplastic activity of

compositions containing NDGA and zinc ions in clinical studies on human patients with basal cell epithelioma.

Compositions as set forth in Table 9 suitable for topical application were prepared:

TABLE 10

Composition Compounds	A	B	C	D	E
zinc chloride	29.8	1.0	5.0	10.0	20.0
NDGA	4.6	4.6	4.6	4.6	4.6
EDTA	14.7	0.49	2.47	4.93	0
BHT	1.1	1.1	1.1	1.1	0
stearyl alcohol	0.5	0.5	0.5	0.5	0.5
H_2O	18.3	18.3	18.3	18.3	18.3
Pego 400	26.4	26.4	26.4	26.3	26.4
Pego 3350	4.5	4.5	4.5	4.5	4.5

The water was heated to about 80-90°C with stirring, and zinc chloride was added. The EDTA was next added with mixing until dissolved. In a separate container the polyethylene glycol 400 was heated to about 80-90°C with stirring, the NDGA was added thereto, then the BHT, and this mixture was added to the zinc chloride-EDTA solution with stirring. The entire mixture was then cooled to about room temperature and passed through a number 3 roller mill until smooth. The polyethylene glycol 3350 was then heated to about 80-90°C and the milled ingredients added thereto with mixing.

The surface of the lesions were tape stripped prior to each application. The test medication was applied directly to the lesion with a coating approximately 2mm thick, and covered with a dressing. After a minimum of

seven (7) days, a second application was applied at the discretion of the investigator. The dose ranged from 20-350 mg/cm^2 with as much as 500 mg/cm^2 utilized for deep tumors. To determine the effect of the test compound on the malignant neoplasma, an excisional biopsy was obtained 30 days after the initial treatment.

Of the fifty seven patients with basal cell epithelioma who were treated with compositions A, B, C or D, twenty showed negative biopsies, i.e., no evidence of tumor, at the conclusion of the treatment period.

EXAMPLE 11

Fifty-nine (59) human patients with actinic keratosis were treated with NDGA plus zinc containing compositions B, C, or D as in Example 9. The test medication was applied directly to the lesion with a coating of approximatley 2 mm and confined to the lesion margin. A dressing was applied to the lesion. A visual examination and measurement of the lesion was performed 7 and 14 days following the initial treatment. At the discretion of the investigator, a second treatment with the same test compound was applied. In order to determine whether the test compound eradicated the premalignant neoplasm, a punch biopsy was obtained 30-60 days after the initial treatment. If the biopsy report was negative, i.e., no tumor, the patient was examined every 6 months for a period of 12 months. If the biopsy continued to show evidence of actinic keratosis, the patient was withdrawn from the study and treated with conventional therapy.

The fifty nine (59) patients had a total of 61 lesions. After treatment with the NDGA plus zinc salt compositions, thirty two of the lesions showed negative biopsies, i.e., there was no evidence of actinic

keratosis.

EXAMPLE 12

Canine patients with various tumor lesions were treated with compositions A, C, D or E of Example 9. The animals were restrained from movement for two hours physically or with sedatives (e.g. 0.03 mg oxymorphone/lb.sq with atropine sulfate). After clipping, washing and measuring the tumor size, the skin surface was abraded until bleeding occurred. To enhance the penetration of the test compositions for large or subdermal tumors, a 20 or 22 gauge needle was used to puncture the tumor. After blotting the skin dry of blood, the tumor site was covered with a 1-2 mm coating of the test composition extending 5 mm peripherially. After 2 hours, the compound was wiped off and the area gently cleansed. The test composition was applied up to three times within a two-week interval or until the tumor cleared. The results of the canine studies are given in Table 11, and show that in canine patients, seven of the twenty four animals showed complete remissions, and another four showed partial remission.

concentrations desired and allowed to cool with vigorous mixing. Any further dilution to achieve desired wt/wt % was achieved by adding Pego 400. When an ingredient was omitted from a particular composition, the amount of the missing ingredient was supplied by adding additional Pego 400. Wt/wt % of compositions utilized in this experiment are given below.

Test Composition

Ingredient in wt/wt %	1	2	3
$ZnCl_2$	4.3	4.3	4.3
Purified water	2.6	2.6	2.6
EDTA	-	2.1	2.1
NDGA	0.66	0.66	0.66
BHT	0	0.66	0
Pego 400	91.04	88.28	88.94
Pego Base	1.4	1.4	1.4

The test compositions were tested in five athymic mice implanted with human breast adenocarcinoma, MX-1. Results are given in Table 4 and confirm the activity of these combinations of the phenolic butane, NDGA, and zinc ions.

TABLE 5

Test Composition	Tumor Free at 60 days	Premature Death	Tumor at Death	Tumor Recurrence
1	4	1	0	0
2	4	0	1	1
3	5	0	0	0

EXAMPLE 6

In order to demonstrate the activity and use of zinc

ions from other salts, two test compositions were prepared according to the procedure previously described. In these, the zinc chloride was replaced by zinc iodide and zinc bromide. Concentrations of the ingredients are given below in wt/wt percent.

	Test Composition	
Ingredient	1	2
BHT	0.65	0.72
EDTA	2.1	2.3
NDGA	0.98	1.1
ZnI_2	3.9	--
$ZnBr_2$	--	4.3
H_2O	2.6	2.9
Pego Base	1.4	0
Pego 400	88.37	88.68

The two compositions were tested for antitumor activity against human breast adenocarcinoma, MX-1, grown in five athymic mice as previously described. The results are given in Table 5.

TABLE 6

Test Composition	Tumor Free at 60 days	Premature Death	Tumor at Death	Tumor Recurrence
1	4	1	0	0
2	4	0	1	0

EXAMPLE 7

A test composition of NDGA plus zinc chloride was investigated for and found to possess antineoplastic activity against xenografts of the following human cancers: lung squamous cell carcinoma, LX-1; breast adenocarcinoma, MX-1; renal cell cancer, RX-1; brain

cancer (glioma); melanoma; and colon cancer, CX-1. The test composition with the approximate wt/wt percentages given below was prepared according to the procedure previously described in Example 4. The control composition was Pego 400.

Ingredient	Test Composition 1	Control
BHT	0.16	-
EDTA	2.10	-
NDGA	0.66	-
$ZnCl_2$	4.26	-
H_2O	2.62	-
Pego Base	1.43	-
Pego 400	88.77	100

The composition was then tested for its effect on human tumors of varying origin implanted in athymic mice as previously described. Generally, there were ten mice in each group tested with Pego 400 control. Instances in which the number of mice varied are specifically indicated.

Results are given in Table 7.

TABLE 7

Tumor Type	Test Composition	Tumor Free at 60 Days	Premature Death	Tumor At Death	Recurrence
LX-1 (lung)	1	8	0	2	0
	control	0	0	5	0
MX-1 (breast)	1	8	0	2	1
	control	0	0	2	0
RX-1 (Renal)	1	8	1	1	0
	control	0	1	5	0
Glioma (Brain)	1	6	0	0	0
	control	0	0	2	0
Melanoma	1	10	0	0	0
	control	0	0	5	0
CX-1 (Colon)	1	8	1	2	0
	control	0	0	5	0

EXAMPLE 8

A number of catecholic butane compositions were formulated into test compositions according to the following general method, and tested for activity against human breast adenocarcinoma, MX-1.

Zinc chloride was dissolved in Pego 400 to prepare a stock solution. The amount of organic compound required to give the final concentration given below was separately dissolved in Pego 400.

The two solutions were mixed to give a final concentration in each test composition of zinc chloride at 0.69 wt/wt % and each organic compound at a molar concentration equivalent to 4.4 wt/wt % of NDGA.

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The test compositions in Table 7 were tested for their effectiveness as antitumor agents against xenografts of the human breast adenocarcinoma, MX-1, grown in athymic mice. They were administered to five animals by intratumor injection. Animals were administered 0.05 ml of test composition unless indicated otherwise.

TABLE 8

Animal Organic Compounds	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
Pego control	0	0	5	0
NDGA	4	1	0	0
d,l NDGA	5	0	0	0
NDGA Tetracetate	4	0	1	0
NDGA Tetrapropionate	4	0	1	1
1,4-bis(3' -methoxy-4' -hydroxyphenyl Butane	2	0	3	0
1,4-bis(3' -methoxy-4' -hydroxyphenyl)-2, 3-dimethyl butane	4	0	1	0
1-(3',4'-dihydroxyphenyl) -4-(2',3',4'-trihydroxy-phenyl)-butane	2	1	3	0
1-(3',4'-dihydroxyphenyl) -4(3',4',5'-trihydroxy-phenyl)-butane	3	0	2	1
1-(3',4',-dihydroxyphenyl) -4-(2',5'-dihydroxyphenyl) -butane	5	0	0	0
1-(3',4'-dihydroxyphenyl) -4-phenyl butane	3	1	1	0
1-(3',4'-dihydroxphenyl) -4-(2',4'-dihydroxyphenyl) -butane	2	0	3	3

EXAMPLE 9

Various zinc salts were tested in combination with NDGA to determine the effectiveness of the compositions according to the invention against xenografts of human breast adenocarcinoma, MX-1, grown in groups of five athymic mice.

The tumors were implanted subcutaneously in the left flank of the mice and the tumors were allowed to grow until they reached an approximate size of between 25 and 100 mm^2 (length x width). The mice were given a single 0.010 ml intratumor injection of the test composition. The concentration of the various metal salts in the test compositions was 0.73% (wt/wt) metal salt and 1.0% (wt/wt) NDGA, in a PEGO 400 base. The results of these test compositions are summarized in Table 8.

TABLE 9

Test Compound	Tumor Free at 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
$ZnCl_2$	4	0	3	3
$ZnSO_4 \cdot 7H_2O$	2	0	3	3
$ZnBr_2$	2	0	3	3
Zn Acetate $\cdot 2H_2O$	2	0	3	3
$Zn(NO_3)_2 \cdot 6H_2O$	3	1	1	1
$ZnCl_2$ (without NDGA)	1	0	19	19

In a separate trial, solubilized zinc gluconate demonstrated efficacious results in the *in vitro* inhibition of clonogenic human lung tumor cells (LX-T) when combined with NDGA.

EXAMPLE 10

This example describes the antineoplastic activity of

compositions containing NDGA and zinc ions in clinical studies on human patients with basal cell epithelioma.

Compositions as set forth in Table 9 suitable for topical application were prepared:

TABLE 10

Composition Compounds	A	B	C	D	E
zinc chloride	29.8	1.0	5.0	10.0	20.0
NDGA	4.6	4.6	4.6	4.6	4.6
EDTA	14.7	0.49	2.47	4.93	0
BHT	1.1	1.1	1.1	1.1	0
stearyl alcohol	0.5	0.5	0.5	0.5	0.5
H_2O	18.3	18.3	18.3	18.3	18.3
Pego 400	26.4	26.4	26.4	26.3	26.4
Pego 3350	4.5	4.5	4.5	4.5	4.5

The water was heated to about 80-90°C with stirring, and zinc chloride was added. The EDTA was next added with mixing until dissolved. In a separate container the polyethylene glycol 400 was heated to about 80-90°C with stirring, the NDGA was added thereto, then the BHT, and this mixture was added to the zinc chloride-EDTA solution with stirring. The entire mixture was then cooled to about room temperature and passed through a number 3 roller mill until smooth. The polyethylene glycol 3350 was then heated to about 80-90°C and the milled ingredients added thereto with mixing.

The surface of the lesions were tape stripped prior to each application. The test medication was applied directly to the lesion with a coating approximately 2mm thick, and covered with a dressing. After a minimum of

seven (7) days, a second application was applied at the discretion of the investigator. The dose ranged from 20-350 mg/cm^2 with as much as 500 mg/cm^2 utilized for deep tumors. To determine the effect of the test compound on the malignant neoplasma, an excisional biopsy was obtained 30 days after the initial treatment.

Of the fifty seven patients with basal cell epithelioma who were treated with compositions A, B, C or D, twenty showed negative biopsies, i.e., no evidence of tumor, at the conclusion of the treatment period.

EXAMPLE 11

Fifty-nine (59) human patients with actinic keratosis were treated with NDGA plus zinc containing compositions B, C, or D as in Example 9. The test medication was applied directly to the lesion with a coating of approximatley 2 mm and confined to the lesion margin. A dressing was applied to the lesion. A visual examination and measurement of the lesion was performed 7 and 14 days following the initial treatment. At the discretion of the investigator, a second treatment with the same test compound was applied. In order to determine whether the test compound eradicated the premalignant neoplasm, a punch biopsy was obtained 30-60 days after the initial treatment. If the biopsy report was negative, i.e., no tumor, the patient was examined every 6 months for a period of 12 months. If the biopsy continued to show evidence of actinic keratosis, the patient was withdrawn from the study and treated with conventional therapy.

The fifty nine (59) patients had a total of 61 lesions. After treatment with the NDGA plus zinc salt compositions, thirty two of the lesions showed negative biopsies, i.e., there was no evidence of actinic

keratosis.

EXAMPLE 12

Canine patients with various tumor lesions were treated with compositions A, C, D or E of Example 9. The animals were restrained from movement for two hours physically or with sedatives (e.g. 0.03 mg oxymorphone/lb.sq with atropine sulfate). After clipping, washing and measuring the tumor size, the skin surface was abraded until bleeding occurred. To enhance the penetration of the test compositions for large or subdermal tumors, a 20 or 22 gauge needle was used to puncture the tumor. After blotting the skin dry of blood, the tumor site was covered with a 1-2 mm coating of the test composition extending 5 mm peripherially. After 2 hours, the compound was wiped off and the area gently cleansed. The test composition was applied up to three times within a two-week interval or until the tumor cleared. The results of the canine studies are given in Table 11, and show that in canine patients, seven of the twenty four animals showed complete remissions, and another four showed partial remission.

TABLE 12

	Test Composition	No. Animal Tested	Cure	Partial Effect	No Effect
Mast cell tumors	A	3	1	1	1
Mast cell tumors	C,D	6	-	2	4
Mast Cell tumors	E	2	1	-	1
Squamous cell carcinoma A	1	1	-	-	
Mammary Adenoma	A	2	1	-	1
Perianal Adenoma	A	7	1	1	5
Perianal Adenitis	A	1	-	-	1
Perianal Cyst (Benign)	A	1	1	-	-
Basal Cell Carcinoma	A	1	1	-	-
Totals		24	7	4	13

EXAMPLE 13

Equine patients with various tumor lesions were treated with compositions A, C, D, or E of Example 9. Melanoma, sarcoid and squamous cell carcinoma lesions were removed to skin level by surgical debulking; for papillomas, the lesion tips were removed. After hemostasis, the tumor site was covered liberally with the test compound extending 5 mm peripherially. Two weeks later, the crust was removed, the lesion area abraded and the test compound applied topically. After an additional two weeks, any crust was again removed from the lesion and the area abraded. The same test compound was again applied topically. Four weeks later, a biopsy of the lesion area was performed. The results of the equine studies in Table 12, show that NDGA plus zinc salt compositions show good activity against the tumor lesions in equine patients. The high activity of the composition against Papillomas, known to have viral

components, indicated the activity of these compositions.

TABLE 13

Lesion	Test Composition	No. Animals Tested	Cure	Partial	No Effect
Papillomas	A	4	4	-	-
Melanoma	A	4	3	-	1
Squamous Cell Car.	A	3	2	1	-
Sarcoid	A	5	4	1	-
Sarcoid	C or D	6	1	2	3
Sarcoid	E	5	5	-	-
		27	19	4	4

EXAMPLE 14

The in vivo antitumor effect of the interaction of NDGA and $ZnCl_2$ at various ratios was determined against MX-1 (human breast adenocarcinoma) cells.

Male or female athymic Balb/c mice, six to eight weeks of age and weighing 20 to 35 grams were used. MX-1 cells were cultured in the standard RPMI-1640 media and implanted subcutaneously in the flank of the nude mice in order to propagate the tumor line. Nude mice were implanted with 25 mg of the MX-1 solid tumor fragments. Tumors which reached the 25-100 mm^2 range were used for the experiment. 0.1 ml of the test compound was injected directly into the tumor. The tumors were measured periodically to determine their weight calculated by using half the product of the length times the width times the height of the tumor. The procedure was repeated at regular intervals until 60 days after

the initial treatment or all mice had died. Mice which showed no evidence of tumors were kept for 60 days to evaluate the potential for tumor recurrence, at which time tumor characteristics, if any, were recorded. Table 13 contains the results of the experiments using mixtures of NDGA and $ZnCl_2$ as well as the results of experiments with NDGA alone or with $ZnCl_2$ alone.

The effective doses (ED_x) at different response levels (x), determined in micromoles for $ZnCl_2$ alone, NDGA alone, and for the combination of $ZnCl_2$ in different molar ratios with NDGA are provided in Table 13.

The significant reduction in amount of either NDGA or zinc chloride required when given in combination is evident from the data. It is also seen that the total amount required for ED_x doses of the composition made up of NDGA plus zinc chloride is significantly less than the ED_x dose of NDGA or zinc chloride alone.

TABLE 14

MICROMOLES

	ED_{50}	ED_{75}	ED_{90}	ED_{95}
NDGA	13.6	25.7	48.3	74.3
$ZnCl_2$	15.7	22.2	31.6	40.1
NDGA (1:1)[1] $+ZnCl_2$	5.7	8.8	13.6	18.2
(1:2)[2]	4.6	6.2	8.4	10.4
(1:2)[3]	2.3	3.1	4.2	5.2

1 Calc'd as micromoles NDGA or $ZnCl_2$.
2 Calc'd as micromoles $ZnCl_2$
3 Calc'd as micromoles NDGA

EXAMPLE 15

Experiments were carried out indicating that zinc acts to stabilize the radical intermediate formed during oxidation of NDGA, thereby effectively stabilizing the NDGA and allowing it to exert its effect over a longer period of time before it is oxidatively inactivated.

Aqueous ethanolic solutions of NDGA with and without various metal salts at pH 4, 7, and 10 were analyzed in an ESR spectrometer for the presence of free radical ion.

The maximum peak height to minimum peak height of the ESR signal was measure over time. The reduction in ESR with time was used as a measure of free radical decay. The slope of free radical decay normalized to that of 3-hydroxytyrosine (DOPA) provided a measure of the relative rate constant of semiquinone free radical decay from NDGA.

The various rate constants, K_d, are given in Table 14.

EXAMPLE 16

To 36.7 grams of powdered Larrea divaricata were added 24.5 grams of powdered rosehips and the mixture was blended in a blender for 5 minutes. The blended mixture was then mixed with 100 milliliters of an aqueous solution containing 185.9 grams zinc chloride to form a paste. The paste was allowed to stand at room temperature for 24 hours. Thereafter, it was stirred and then placed in a screw-capped glass container. The container was placed in a humidified oven at 40°C for 5 days. This incubated paste was then suspended in 500 milliliters of triple distilled water and shaken at room temperature for 24 hours on a reciprocating shaker. The zinc chloride extract solution was then evaporated to near dryness on a rotary evaporator at 90°C under reduced pressure. A sufficient quantity of this dried zinc chloride extract was added to 120 grams of an ointment base consisting of 10% (w/w) stearyl alcohol and 90% (w/w) polyethylene glycol to obtain an ointment containing 70% (w/w) of the extract.

EXAMPLE 17

A sufficient quanitity of the paste of Example 17 was added to sterile deionized water to obtain a concentration of 10 grams per 100 milliliters of water. The aqueous mixture was thoroughly shaken for one hour on a reciprocating shaker, then the aqueous suspension was filtered through Whatman #1 filter paper in a Buchner funnel. The filtrate, an aqueous suspension, was used to irrigate wounds in the treatment of osteomyelitis.

EXAMPLE 18

Five selected human patients with osteomyelitis of duration of from several months to several years were treated topically with the solution of Example 17 and/or the paste of Example 16. In all instances, the osteomy-

elitis had been unresponsive to conventional treatment, and upon the application of the preparation, the patients received no other conventional therapy except as indicated. In some cases, the wounds were debrided, prior to the application of the preparation. Upon application of the preparation, most patients experienced pain and a burning sensation over the area which had been treated and some patients additionally experienced swelling and inflamation. One patient experienced severe nausea after an application of the preparation.

Summaries, histories, and treatment are given below in Table 18. With respect to patient one, the disease process was so extensive that prior to treatment, a partial amputation of his foot was indicated. With respect to patient four, the disease process was so extensive as to cause the exposure of the extensor tendons which normally necessitates their cutting. Moreover, as a result of the destruction of the bones of the ankle and foot, the possibility of an ankle fusion was considered; however, neither of these procedures was required as the patient became ambulatory without the assistance of either a cane or crutches within six months of the beginning of the treatment with the preparation.

TABLE 18

Patient	Diagonosis	Culture	Previous Treatment	Duration of Condition	Number of Treatments	Time Required for Healing of Lesion
1 (62 year old male)	Chronic diabetic ulcer of left foot with osteomyelitis extending down to the metatarsal head capsule, involving the flexor tendon of the fourth toe	Hemolytic *Staphylcoccus aureus* coagulase positive	Antibiotics with no response	Several months	2;13 days apart	1-1/2 months
2 (59 year old male)	Chronic ulceration of lateral aspect of the proximal fibula	*Staphylcoccus aureus* coagulase positive	Multiple skin graftings; multiple antibiotics	Several years	3;19 and 23 days apart	3 months
3 (63 year old male)	Chronic osteomyelitis of left ankle and distal tibia	Hemolytic *Staphylcoccus aureus* coagulas positive	Recent treatment with Betadine soaks	35 years	4;over a 3-month period (first two with the solution and last two with the paste)	9-1/2 months for complete recovery

TABLE 1B (Cont)

Patient	Diagonosis	Culture	Previous Treatment	Duration of Condition	Number of Treatments	Time Required for Healing of Lesion
4 (70 year old female)	Ulcer of the left foot with necrosis, drainage, destruction of the bones of the foot and ankle initiated by a bite from a brown recluse spider	Hemolytic Staphylcoccus aureus coagulase positive	Antibiotics and soaks	7 months	2;5 days apart	1-1/2 months for lesion; after 6 months able to walk without crutches
5 (68 year old male)	Stasis ulcers of lower left extremity due to circulatory impairment	-	Steroid cream and ointment	unknown	2;9 days apart. Treated with a diuretic and soaks were applied to the area to reduce swelling apparently caused by the treatment.	2-1/2 months

EXAMPLE 19

Fifteen older dogs having perianal adenomas were treated topically with the ointment of Example 16 having a strength of 55% (w/w). The normal treatment for such a condition is surgery; however, these older dogs were poor surgical risks. The tumor of each dog was biopsied and the ointment was applied topically into the biopsied incision. The duration of treatment varied depending upon the severity of the adenoma. Dogs with simple circumscribed adenomas required only one treatment. The dogs with more advanced adenomas generally required more than one treatment which were given three to five days apart. The treatment was successful in thirteen of the fifteen dogs. The treatment was not successful in two of the dogs which had extremely advanced cases of perianal adenomas.

EXAMPLE 20

An incubated paste of rosehips, zinc chloride and Larrea divaricata prepared in accordance with the method of Example 16 was placed into gelatin capsules such that each capsule contained 200 mg of the paste. A patient with glioastrocytoma was treated orally with these capsules. Prior to this treatment the patient had a resistant tumor which displaced the cranium and protruded from the right lateral aspect of the skull; the protrusion measured 7 x 7 mm. The patient received 200 mg oral doses four times a day for a total daily dose of 800 mg. Observable and subjective improvement occurred within seven days; in 71 days the tumor had become cystic and lysed. The protuberance of the skull was reduced to near normal dimensions by repeated aspirations of the clear amber cystic tumor fluid. The patient has been maintained on the 200 mg capsules given four times daily and has remained symptom free for over 18 months.

Eleven cutaneous ulcers in eight human patients were treated with a formulation containing 5% zinc chloride and 4.6% NDGA w/w. If excessive necrotic material was present, debridement of non-viable and foreign material was performed either surgically or with wet-dry dressings prior to treatment.

The test compound was applied directly to the cutaneous ulcer in an amount sufficient to cover the visual margins of the ulcer. The treated ulcer was then covered with a loose dressing and the patient advised against washing the treated area for a reasonable period of time. A scab or crust was observed to form on the surface of the ulcer. Normally within two weeks the crust had loosened to where it was sluffed off or could be readily removed. It was observed that granulation of the tissue in the ulcer had occurred in those ulcers which slowed clinical improvement. A second treatment with the Compound was applied after removal of the crust. The patient was visually examined and the ulcer measured within two weeks after the initial treatment. Thereafter, the patient returned twice a month for two months for a visual examination and measurement of the ulcer. Of the eleven (11) treated lesions, seven (7) were clinically improved.

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EXAMPLE 22

Six (6) Kaposi's sarcomas in human patients were treated with Compound A, (a formulation containing 29.8% zinc chloride and 4.6% NDGA w/w) which was applied directly to the lesion with a thickness of approximately 2 mm and confined to the visual margins of the lesion. The lesion was then covered with a dressing and the patient advised against washing the treated area for a reasonable period of time. The patient was visually examined 1, 2, 3, 7 and 14 days after the initial treatment. If possible, accurate measurements of the lesion were taken and recorded. A second application of Compound A was applied as deemed necessary. After 14 days, a biopsy was obtained if the lesion appeared clinically improved. If the biopsy continued to show evidence of Kaposi's sarcoma or if the lesion was not clinically improved by the 14th day after the initial treatment, the patient was withdrawn from the study. Due to the serious nature of the disease, the 14-day time period was arbitrarily chosen as the termination point in order to provide patients who had not clinically improved the opportunity to pursue other methods of treatment regardless of biopsy results.

EXAMPLE 23

Cultures of representative microorganisms which included Gram negative and Gram positive bacteria, yeasts and molds were prepared to assess the effect of composition A of Example 10, as well as its separate components, on the survival and/or growth of the microorganisms. The microorganisms and the culture media used are given below.

- Streptococcus sp., Group C, ATCC 9342 (Stp. Pyogenes, Lancefield Group A).
- Staphylococcus aureus (penicillin sensitive), ATCC 9144
- Staphylococcus aureus (penicillin resistant), ATCC 13301
- Escerichia coli, ATCC 11229
- Proteus mirabilis, ATCC 4675
- Mycobacterium smegmatis, ATCC 20
- Bacteroides fragilis, ATCC 23745
- Candida albicans, ATCC 28366
- Candida kruseri, ATCC 2159
- Trichophyton mentagrophytes, ATCC 9533
- Microsporum canis, ATCC 9084

All of the bacterial species, including M. smegmatis, were found to grow well in tryptic soy broth with dextrose (TSB). Good growth was also obtained with the yeast species in this medium. Although the fungal species grew in TSB, they grew somewhat better in Sabouruad's broth (SAB), and for the broth dilution tests with T. mentagrophytes and M. canis Sabouruad's was used. For spore production the fungal species were grown on malt-soil extract agar.

A series of tests were devised to determine the effect of direct exposure of the microorganisms to the test compositions. The tests were conducted according to the following general procedure.

Sterile tubes of a growth medium (broth) appropriate for the bacteria, yeast, or mold under test were inoculated and allowed to grow until the tube exhibited the maximum turbidity that could be expected for the particular species. For most bacteria and yeasts this time was 24 hours. For molds the procedure was different in that the fungal species were inoculated onto the surface of a malt extract-soil extract agar slant and allowed to grow at room temperature until a heavy mycelial growth with heavy spore production was observed. At this time the spores were washed from the mycelia with sterile water and agitation using a vortex mixer. The spore suspension was filtered through four layers of sterile cheesecloth into sterile tubes. The spore suspensions were handled from this point in the same manner as broth suspensions of bacteria or yeasts.

One milliliter of the bacterial, yeast, or mold spore suspension was transferred to a sterile 12-ml glass, conical, centrifuge tube covered with a sterile cap and centrifuged at 3,000 rpm for 15 min. Centrifugation was done at room temperature using a benchtop, angle-head, clinical centrifuge (Clay-Adams). After the bacteria, yeast, or mold spores were pelleted, the supernatant fluid was decanted and the tubes inverted over paper saturated with a biocide placed in a bacteriological hood.

The pellets in the centrifuge tubes were then mixed with 1 gram of the undiluted test material and allowed to remain in contact for 2 hours at 37°C for the bacteria and yeasts and at 25°C for the mold spores. At the end of the contact time, the test mixture was diluted 1 to 10 with growth medium (TSB or SAB broth). Additional serial

dilutions were made from the initial dilution up to 1 x 10^{-9}. Each material was tested in triplicate. The controls, which consisted of the microbial cells incubated with 1 gram of mineral oil, were diluted in the same way. All dilutions of both test materials and controls were then incubated at an appropriate temperature of 37°C for bacteria and yeasts and 25°C for molds to allow for growth of any viable cells present.

All bacterial species except M. smegmatis were incubated for 48 hours; M. smegmatis was incubated for 7 days. Yeast tests were incubated 48 hours. Molds were incubated for 10 days. For a determination of growth response, growth in tubes containing test compositions was compared to the growth in a mineral oil control at an equivalent dilution. Growth was indicated by turbidity in the broth medium.

Results of the direct exposure tests are given in Table 23.

TABLE 23

Direct Exposure Tests
(Organism Exposed to the Composition
for 2 Hours Prior to Dilution in Test Broth)

	Test Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
	pH of Test Broth		5.5	6.8	7.0	7.0	7.0	7.0	7.0	7.0
	Precipitation of Test Compound	heavy ppt	heavy ppt	ppt	slight ppt	-	-	-	0	-
Streptococcus pyogenes	Growth in Test Broth	0/3 [1]	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Growth in Mineral Oil Control	+ [2]	+	+	+	+	+	+	+	+
Staphylococcus aureus (penicillin sensitive)	Growth in Test Broth	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Growth in Mineral Oil Control	+	+	+	+	+	+	+	+	+
Escherichia coli	Growth in Test Broth	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Growth in Mineral Oil Control	+	+	+	+	+	+	+	+	+

1. No growth shown in any of the three tubes at that dilution.
2. Indicates growth of cells occurred.

TABLE 23 (Cont.)

	Test Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
	pH of Test Broth		5.5	6.8	7.0	7.0	7.0	7.0	7.0	7.0
	Precipitation of Test Compound	heavy ppt	heavy ppt	ppt	slight ppt	-	-	-	0	-
Mycobacterium smegmatis	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0	0	0	0
	Growth in Mineral Oil Control	-	+	+	+	0	0	0	0	0
Bacteriodes fragilis	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0	0	0	0
	Growth in Mineral Oil Control	-	+	+	+	+	0	0	0	0
Candida albicans	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0
	Growth in Mineral Oil Conrol	-	+	+	+	+	+	+	+	0
Microsporum canis	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0	0	0	0
	Growth in Mineral Oil Conrol	-	+	+	+	+	0	0	0	0

EXAMPLE 24

Tests were conducted on the effect of the direct exposure of representative microorganisms to several separate components of the compositions of Example 10. Pego base alone was tested in one series of evaluations to determine whether or not inhibition by this carrier would have to be considered in evaluating the results of the individual ingredients dissolved in it.

In order to better approximate the effects of pego base in the Example 10 formulations, the amount of polyethylene glycol present in the formulation was calculated. The pure base material was then diluted with water to this concentration. Mineral oil was used as a positive control.

Nordihydroguaiaretic acid (NDGA) and desmethyl NDGA (DM-NDGA) diluted in pego base were also tested for inhibitory properties against representative gram-negative and gram-positive bacteria and yeasts covering the spectrum of microorganisms used in these tests.

The initial concentration of the compounds tested was equivalent to the amount present in the composition, and the general procedure outlined in Example 23 was followed. After a 2-hour exposure of the microorganisms to this initial concentration, progressive 1 to 10 serial dilutions of the mixture were made to assess viability of any microorganisms present. Results are shown in Table 24.

TABLE 24

Growth of Selected Microorganisms Following Direct Exposure Tests to NDGA, Demethyl NDGA, PEGO

Microorganism Tested	NDGA[1] micrograms/ml				DH-NDGA[1] micrograms/ml				PEGO[1] micrograms/ml				Mineral Oil micrograms/ml			
Concentration of Test Material	4,600	460	46	4.6	1,100	110	11	1.1	310	31	3.1	0.31	500	50	5.0	0.5
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Streptococcus pyogenes	0	0	0	0	0	0	0	0	2^+	2^+	2^+	2^+	4^+	4^+	4^+	4^+
Escherichia coli	2^+[2]	4^+[3]	4^+[4]	4^+	0	0	4^+	0	4^+	4^+	4^+	4^+	4^+	4^+	4^+	4^+
Staphylococcus aureus (Penicillin Resistant)	0	0	4^+	4^+	0	0	4^+	4^+	2^+	4^+	4^+	4^+	4^+	4^+	4^+	4^+
Candida albicans	0	0	2^+	2^+	±	1^+	3^+	3^+	3^+	3^+	3^+	3^+	4^+	4^+	4^+	

1/ All test media at all dilutions were at pH 7.3.
2/ A heavy precipitate formed and bacterial turbidity was estimated; there was obvious growth with heavy production of gas.
3/ Moderate precipitate.
4/ Light precipitate.

EXAMPLE 25

A combination of EDTA (ethylenediaminetetraacetic acid) and zinc chloride in pego base at the concentration in which these components are present in composition A of Example 10 was tested for its effect on the viability of representative microorganisms. All organisms given in Example 23 were tested except Candida kruseri, and Microsporum canis.

The test procedure followed was that generally described in Example 23. The initial test composition of EDTA/zinc to which the microorganisms were exposed for 2 hours of direct contact had a pH of about 2.0. None of the organisms tested retained viability after exposure to this test mixture. All mineral oil controls showed abundant (4^+) growth.

In every test a heavy precipitate formed when the test mixture was diluted to 10^{-2}. The pH of the 10^{-2} dilution was 4.75. No precipitate formed at the 10^{-1} dilution (pH 1.8), the 10^{-3} dilution (pH 6.60) or the 10^{-4} dilution (pH 7.0).

EXAMPLE 26

A test was conducted to assess the growth of Escherichia coli and Staphylococcus aureus in broth containing composition A of Example 10, NDGA or desmethyl NDGA diluted in glycerol. Test parameters and results are given below.

TABLE 49

	Growth (48 hr at 37°C)	
	E. coli	S. aureus
Glycerol		
1 ml in 10 ml TSB	4+	4+
0.1 ml in 10 ml TSB	4+	4+
NDGA in Glycerol		
100 ppm (as NDGA) in TSB	3+	0
1,000 ppm (as NDGA) in TSB	3+	0
Desmethyl NDGA in Glycerol		
100 ppm (as DM-NDGA) in TSB	3+	0
1,000 ppm (as DM-NDGA) in TSB	3+	0
Compound A in Glycerol		
100 ppm	4+ (4.6 ppm NDGA + 1.1 ppm BHT)	4+
1,000 ppm	4+ (46 ppm NDGA + 11 ppm BHT)	0

EXAMPLE 27

A series of broth dilution tests were conducted to assess the effect of composition A of Example 10 and its separate components on the growth of microorganisms. The individual test materials were incorporated into pego base at the concentration in which they are present in the composition for testing. EDTA and zinc chloride were tested together. Each original formulation was diluted 1 to 10 with growth medium, (usually tryptic soy broth with glucose), and subsequent 1 to 10 dilutions were made of the previous dilution usually up to 1×10^{-4}. This test was done with no consideration given to the solubility of the test material when diluted. In all cases, controls consisting of cells in mineral oil diluted in TSB were made to test the effect of the medium on growth. The pH determination of each series of materials was made by testing a duplicate set of tubes that were uninoculated. Each dilution tube containing 10 ml. test broth was inoculated with 0.1 ml. of a 24-hour culture of all test species except M. smegmatis and the mold species. Spore suspensions of fungi (10 days) were used to inoculate the tubes for testing effects on M. canis and T. mentagrophytes, and Sabouraud's broth was used for dilution because the fungal species grew somewhat better in this medium than in TSB. In general, a stationary phase culture of each test species was used.

Results of the broth dilution tests are given in Table 27. The pH values given in the tables apply only to the dilution shown.

The readings of turbidity in the growth media which indicate growth of the microorganism are rated from 0 = no growth, to 4+ = turbidity equal to the control. A 4+ reading for one microbial culture does not mean that the turbidity of that culture was the same as a 4+ reading

for any other culture. A 4+ reading means that turbidity in the tubes of a particular test was equal to the turbidity of the appropriate control at the dilution compared.

TABLE 27

Broth Dilution Tests

Test Microorganism	Test Compound	Compound A				Control Pego Base			
	Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
	Broth pH	2.6	5.2	6.6	6.6	7.1	7.1	7.1	7.1
Streptococcus pyogenes		0	0	0	4+	4+	4+	4+	4+
Staphylococcus aureus (Pen. Resistant)		0	0	0	4+	4+	4+	4+	4+
Staphylococcus pyogenes (Pen. Sensitive)		0	0	0	4+	1+	4+	4+	4+
Escherichia coli		0	0	4+	4+	4+	4+	4+	4+
Proteus mirabilis		0	0	4+	4+	4+	4+	4+	4+
Mycobacterium smegmatis		0	0	0	2+	±	4+	4+	4+
Bacteriades fragilis		0	0	0	0	4+	4+	4+	4+
Candida albicans		0	1+	4+	4+	2+	4+	4+	4+
Candida kruseri		0	0	2+	3+	4+	4+	4+	4+
Trichophyton mentagrophytes		0	0	2+	2+	4+	4+	4+	4+
Microsporum canis		0	0	4+	4+	not tested			

TABLE 27 (Cont.)

Broth Dilution Tests

Test Microorganism	Test Compound	NDGA in Pego Base				Desmethyl NDGA in Pego-Base				EDTA Zinc Chloride 1/			
	Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
	Broth pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	1.8	4.75	6.6	7.0
Streptococcus pyogenes		2^+	3^+	4^+	4^+	0	4^+	4^+	4^+	0	0	0	4^+
Staphylococcus aureus (Pen. Resistant)		0	4^+	4^+	4^+	0	2^+	4^+	4^+	0	0	0	4^+
Staphylococcus pyogenes (Pen. Sensitive)		0	4^+	4^+	4^+	0	1^+	4^+	4^+	0	±	4^+	4^+
Escherichia coli		±	4^+	4^+	4^+	1^+	4^+	4^+	4^+	0	0	4^+	4^+
Proteus mirabilis		4^+	4^+	4^+	4^+	4^+	4^+	4^+	4^+	0	0	4^+	4^+
Mycobacterium smegmatis		0	0	4^+	4^+	0	1^+	4^+	4^+	0	0	0	2^+
Bacteriades fragilis		0	0	2^+	2^+	0	0	1^+	1^+	0	0	0	2^+
Candida albicans		1^+	2^+	3^+	4^+	1^+	2^+	4^+	4^+	0	0	4^+	4^+
Candida kruseri		0	0	4^+	4^+	0	2^+	3^+	4^+	not tested			
Trichophyton mentagrophytes		0	0	2^+	2^+	0^+	0^+	2^+	2^+	0	0	2^+	2^+
Microsporum canis		0	0	4^+	4^+	0	0	2^+	2^+	not tested			

1/ At the 10^{-2} dilution, pH 4.75 all tubes had a heavy precipitate. No precipitate formed at the other dilutions.

EXAMPLE 28

Compositions containing NDGA, zinc chloride or a combination of NDGA-Zn were tested for antimicrobial activity individually against seven gram positive and gram negative bacterias, yeasts and molds.

In a preliminary screening test, a vehicle containing 30% polyethylene glycol-200 (PEGO-200) + 0.1% sodium ascorbate in deionized water at various concentrations was shown to exhibit no inhibitory effect on microbial growth and was chosen as the diluent for the test compounds. Stock solutions of the test compounds in 30% PEGO-200/water were prepared at the following weight percent concentrations: 4.6% NDGA + 0.1% ascorbic acid; 5.0% $ZnCl_2$ + 0.1% sodium ascorbate; and 4.6% NDGA + 5.0% $ZnCl_2$ + 0.1% sodium ascorbate. Aliquots of the stock solutions were diluted 1:10 and 1:100 with the 30% PEGO-200 diluent. The stock solutions were further diluted 1:10 with Brain Heart Infusion Agar, which was melted at 45°C prior to the addition of the test solutions. The agar containing the test solutions was then poured into 50 x 90 mm petri dishes and allowed to dry for four hours at room temperature prior to inoculation.

All Brain Heart Infusion slants were started at 35°C anaerobically except for T. mentagrophytes at 27°C and P. acnes at 35°C anaerobically. Those microbial slants incubated at 35°C were subsequently transferred to new slants at 35°C and incubated at the same temperature. All slants were harvested with 1 ml saline containing 0.05% Tween-80 and diluted with saline in the following amounts to be used as working inocula: 1 ml each of E. Coli, P. aeruginosa, S. aureus and B. subtilis was diluted with 99 ml saline; 1 ml of C. albicans and P. acnes was diluted with 9 ml saline; 1 ml of T. mentagrophytes was left undiluted.

One drop of working inocula was added to the petri dishes containing the test compounds and allowed to

absorb into the agar. Uninoculated (control) and inoculated dishes were sealed and incubated in the dark for 5-7 days under the following conditions: P. acnes anaerobically at 35°C, T. mentagrophytes at 27°C and the remaining at 35°C aerobically. The plates were visually observed for microbial growth. Table 28A shows the dose levels and inhibitory effects of the test compounds. Table 28B provides a summary of the results with the test compounds showing the lowest dosage with complete inhibition.

TABLE 28.A

% Active(s) in Agar	E. coli	P. aeruginosa	S. aureus	B. subtilis	P. acnes	C. albicans	T. mentogrophytes
0.46% NDGA	-	-	++	++	++	++	+
0.046% NDGA	-	-	++	++	++	+	-
0.0046% NDGA	-	-	-	-	-	-	-
0.5% $ZnCl_2$	++	++	++	++	++	++	++
0.05% $ZnCl_2$	-	-	-	+	+	-	-
0.005% $ZnCl_2$	-	-	-	-	-	-	-
0.46% NDGA + 0.5% $ZnCl_2$	++	++	++	++	++	++	++
0.046% NDGA + 0.05% $ZnCl_2$	-	-	++	++	++	+	+
0.0046% NDGA + 0.005% $ZnCl_2$	-	-	-	-	++	-	-
Vehicle Control (with 0.1% Sodium Ascorbate)							
0.2% Polyethylene Glycol-200	-	-	-	-	-	-	-
2.0% Polyethylene Glycol-200	-	-	-	-	-	-	-
20.0% Polyethylene Glycol-200	-	-	-	-	-	-	-

Results are found in triplicate samples:

++ = Complete inhibition of growth

+ = Partial inhibition (some growth)

- = Little or no inhibition (good growth)

EXAMPLE 20

A composition containing 5% NDGA plus 10% zinc chloride was tested for antiacne activity. Comedones were induced in both ears of rabbits by daily application of coal tar to the skin of the external ear canal. The right ear of each animal was treated with the test agent daily (5 days) for two weeks. The left ear served as the untreated control. Comedones in the test ears were small compared to those in the control ear. Horny material in the test ears was moderately reduced in 3 out of 5 animals. Peri-comedonal inflammation was significantly less in treated ears compared to test ears treated with vehicle alone.

While there have been described what are presently believed to be preferred embodiments of the invention, it will be apparent to a person skilled in the art that numerous changes can be made in the ingredients, conditions and proportions set forth in the foregoing embodiments without departing from the invention as described herein and as defined in the appended claims.

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NOT TO BE TAKEN INTO

CONSIDERATION FOR THE

PURPOSES OF INTERNATIONAL

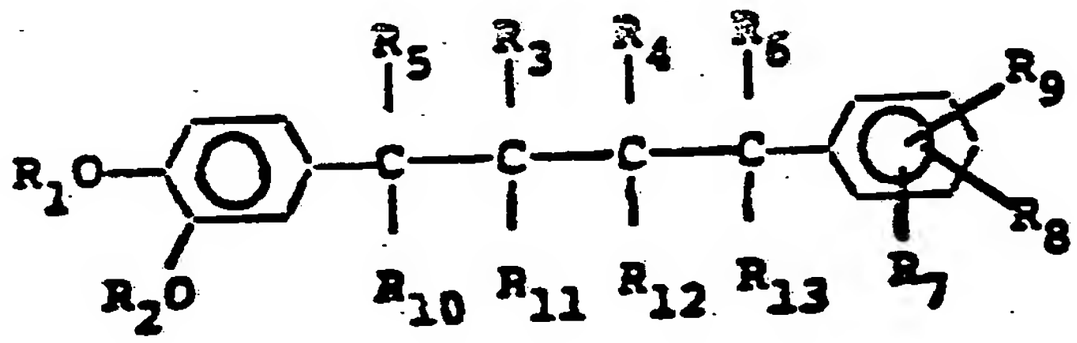
PROCESSING (See Section 309(c)(ii)

OF THE ADMINISTRATIVE INSTRUCTIONS)

15. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the composition of claim 5 wherein said ionic zinc is derived from a pharmaceutically acceptable salt.

16. Pharmaceutical compositions comprising a pharmaceutically acceptable carrier and the composition of claim 7 wherein said ionic zinc is derived from a pharmaceutically acceptable salt.

17. A method for treating solid tumors, which comprises administering to a mammal in need of said treatment an effective amount of a composition comprising ionic zinc and at least one catecholic butane of the formula:



wherein R_1 and R_2 are independently H, lower alkyl or lower acyl;

R_3, R_4, R_5, R_6, R_{10}, R_{11}, R_{12} and R_{13} are independently H or lower alkyl;

R_7, R_8 and R_9 are independently H, hydroxy, lower alkoxy or lower acyloxy; and

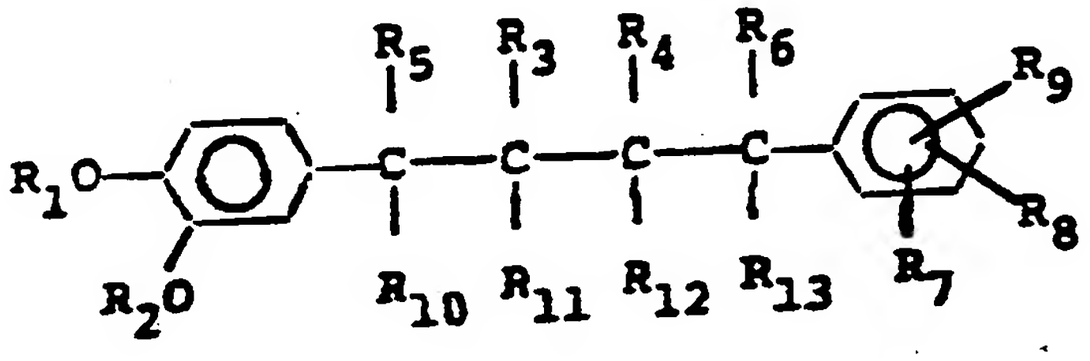
ionic zinc.

18. The method according to claim 17, wherein the catecholic butane is nordihydroguaiaretic acid, and the ionic zinc is a cation of a pharmaceutically acceptable water soluble salt.

19. The method according to claim 18, wherein the composition is administered topically to the solid tumor.

20. The method according to claim 19, wherein 2-20 mg of composition are applied to each cm^2 of solid tumor.

21. A composition comprising at least 1 catecholic butane of the formula:



wherein R_1 and R_2 are independently H, lower alkyl, or lower acyl;

R_3, R_4, R_5, R_6, R_{10}, R_{11}, R_{12} and R_{13} are independently H or lower alkyl;

R_7, R_8 and R_9 are independently H, hydroxy, lower

alkoxy or lower acyloxy.

22. A method for treating solid tumors, which comprises administering to a mammal an effective amount of the composition according to claim 21.

23. A method of promoting the healing of a lesion in the tissue of a mammal which comprises applying to said lesion an amount of a composition according to Claim 1 effective to promote the healing thereof.

24. The method of Claim 23, wherein said composition promotes the granulation of tissue in the surface of the lesion.

25. The method of Claim 23, wherein said lesion results from acnes.

26. The method of Claim 23, wherein said lesion results from osteomyelitis.

27. A method for inhibiting the proliferation of a microorganism said method comprising applying to the situs of growth of said microorganism a proliferation inhibiting amount of the composition of Claim 1.

28. The method of Claim 27, wherein said microorganism is selected from the group consisting of Propionibacterium acnes and Staphylococcus aureus.

29. The method of Claim 28 wherein said catecholic butane comprises 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane and said source of ionic zinc comprises zinc chloride and wherein said catecholic butane and ionic zinc together comprise between about 0.5 and about 80 weight percent of said composition and the molar ratio of said catecholic butane to said ionic zinc is between about 5 to 1 and 1 to 10 with the proviso that said zinc chloride is present in less than an escharotic amount.

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30. A method for enhancing the retention time of a catecholic butane in tissue at the situs of topical application said method comprising applying said catecholic butane as a composition according to Claim 1 which composition contains an amount of ionic zinc effective to enhance said retention time.

31. The method of Claim 30, wherein said source of ionic zinc is zinc chloride, bromide, iodide, nitrate, phosphate, sulfate, acetate, benzoate, citrate, caprylate, gluconate, or a mixture thereof.

32. The method of Claim 30 wherein said catecholic butane is 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane and said source of ionic zinc comprises zinc chloride.

33. A method for enhancing the oxidative stability of a catecholic butane in the composition of Claim 1 said method comprising providing a concentration of said ionic zinc effective to retard the formation of oxidative by-products from said catecholic butane.

34. The method of Claim 33, wherein said ionic zinc is present in an amount sufficient to decrease the decay rate of at least a portion of semiquinone free radicals formed from said catecholic butanes.

35. The method of Claim 34, wherein the molar ratio of said ionic zinc to said catecholic butane is greater than about 1 to 5.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/02549

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL4: A61K 33/30; A61K 31/05
U.S. CL : 425-145; 514-734

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/145; 514/734

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Chemical Abstracts (1907-1986)
Guiaretic Acid
Dihydro and Derivatives; with or without Zinc Chloride

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	N, An Index Of Tumor Chemotherapy published Mar, 1949), pages 10-12, 40 and 41, compound nos. 981-990, Dyer.	17-20
X	N, Cancer Research, Vol. 19, No. 10, part 2 published Nov. 1959, pages 488-494 and 556 compound No. 16440, Leiter et al.	17-22
X	U.S.A. 3,934,034 MANNING published 20 Jan. 1976.	21
A	U.S.A. 4,094,994 SCHOENBERGER ET AL published 13 June 1978	17-22

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
11 Feb. 1987	02 MAR 1987
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Jerome Goldberg

Form PCT/ISA/210 (second sheet) (October 1981)